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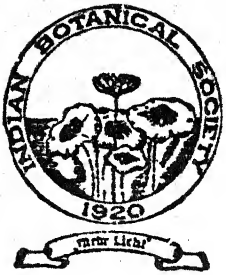

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The Journal of the Indian Botanical Society

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SOME FACTORS AFFECTING THE GROWTH AND SURVIVAL OF *FUSARIUM VASINFECTUM* ATK., THE COTTON WILT PATHOGEN IN THE SOIL, WITH SPECIAL REFERENCE TO MICROBIOLOGICAL ANTAGONISM

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Received for publication on December 8, 1945

Introduction

Numerous studies have been made on the growth of the fungus *Fusarium vasinfectum* Atk., as affected by various factors like temperature, pH, etc., in pure-culture, by a number of workers (Neal, 1927 ; Fikry, 1932 ; Kulkarni, 1934 ; Mitra and Kheswalla, 1935). The media used in these studies have mostly been synthetic agar media or in other cases synthetic liquid media, the growth of the fungus usually being measured in terms of the diameter of the colonies in the former case, and in terms of dry weight of fungal growth in the latter. While pure-culture studies have been useful in many ways, such studies have not taken full cognisance of Fawcett's (1930) well-known assertion that "nature does not work with pure cultures alone," but most frequently with a varied and diverse microfloral complex. Quite recently a vast amount of literature has accumulated on the subject of antagonism which emphasizes the importance of the microbiological factor of the soil, especially in relation to soil-borne diseases and their control.

On the one hand, experiments conducted in this laboratory to grow *Fusarium vasinfectum* in (i) ordinary unsterilised soil, (ii) the same soil (*a*) with addition of steamed rice (powder), (*b*) with addition of steamed rice (powder) and cane-sugar, and (*c*) with addition of Duggar's synthetic nutritive solution, resulted in failure, with *Mucor* sp., and other fungi predominating in all cases. On the other hand,

experiments in which attempts were made to grow the fungus in parallel sterilised treatments all succeeded, better growth being obtained when rice powder, cane-sugar and Duggar's solution were used as extra nutrients. It was, therefore, clear that nutritional deficiencies were not responsible for the failure of the fungus to grow in the unsterilised soil but that the inhibiting factors were indicative of antagonism by the soil microflora.

Further work was, therefore, necessary to explain the failure of the fungus to grow in the unsterilised soil. Accordingly, an investigation of the factors affecting the growth and survival of *Fusarium vasinfectum* in the soil, using the direct microscopic method of Cholodny (1930), was taken up and forms the subject of the present communication.

Experimental

Technique.—A modified Cholodny slide technique was used throughout the course of this investigation. The technique consisted in burying thin films of the pathogen, *i.e.*, *Fusarium vasinfectum* Atk., grown on sterile slides into the soil, and taking them out at intervals for examination. The detailed experimental procedure was as follows : Clean microscope slides preserved in absolute alcohol were used for the purpose. A spore suspension was prepared from a culture of the fungus *Fusarium vasinfectum* grown in Duggar's solution for ten days (incubation temperature 29°–32° C). The spore suspension was added at the rate of 1 c.c. for 10 c.c. of a modified Horne and Mitter's medium, maintained at about 42° C. The spore suspension was mixed well with the medium, and the Cholodny's slides were coated on a limited area of the slide only with this spore-suspension-in-agar and incubated at room temperature in sterile moist chambers in the form of petri dishes. The slides were later on taken out after definite periods of incubation and then buried in soils placed in cylindrical container jars of size 4″ × 3″ which had been previously autoclaved at 15 lbs. pressure for 20 minutes. In the case of sterilised treatments the containers were sterilised along with the soil by autoclaving at 20 lbs. for 2 hrs. The moisture percentage of the soil in all cases was adjusted to 50 by addition of the calculated amount of sterile distilled water. The process of burying the slides and their removal was in essentials that of Conn (1932). This was done as follows : the slides were buried vertically (with their longer edges parallel to the sides of the container) in trenches dug in the soil. Some soil (adjusted to 50% moisture, sterilised or unsterilised and with or without amendments according to the treatment) was then loosely packed all around the slides. The whole operation was done under aseptic conditions. The slides so buried were incubated in the soil for definite periods before they were taken out for examination. In removing the slides, the entire core of soil with the buried slides was first smartly tapped out from the container and then the slides were pulled away from each face of soil in turn, taking care to see that the thin film of microorganisms on the slide was left intact. The staining procedure of Jensen (1934) was found to be most suitable and has been used throughout the course of this investigation. Jensen's staining method was as

follows : "(1) After air-drying and removal of sand-grains, the slide is passed through a Bunsen flame to fix the micro-organisms.—(2) Staining 2–3 minutes with Crystal-violet-ammoniumoxalate-solution (Gram-Hucker).—After washing treatment for 1–2 minutes with Lugol's iodine.—(3) Washing, drying, and differentiation 4–5 minutes with absolute alcohol which is renewed 3–4 times.—(4) Drying and counter-staining 10–12 minutes on water-bath at 60–70° C. with Rose bengale solution after Conn (1932)" (Jensen, 1934, p. 202). Every region of the stained preparations was carefully explored under the microscope, and representative formations were photomicrographed. Some of the photomicrographs are reproduced in Plate V.

Two soils were used* :

(1) A sandy compost soil prepared by mixing sand, red earth and dung manure in the proportion of 2 : 1 : 2. Saturation capacity 33·9 ; pH 6·5 approximately.

(2) A sticky black cotton soil from Udamalpet (Coimbatore Dt., Madras Presidency). Saturation capacity 72·3 ; pH 8 approximately.

Two different strains of *Fusarium vasinfectum* pathogenic to cotton were employed during this investigation : (1) a culture of *Fusarium vasinfectum* pathogenic to cotton kindly sent by Mr. K. M. Thomas, Government Mycologist, Coimbatore. This strain has undergone continued subculturing through several generations ; (2) a strain of *Fusarium vasinfectum* pathogenic to cotton recently isolated by the author in this laboratory from wilted cotton plants from a typically *Fusarium*-wilt-infected field at Udamalpet. The strain resembled Mr. Thomas's type culture in every way, both morphologically and physiologically. The demonstration of the pathogenicity of the isolate to cotton, coupled with the historical interest which attaches to Udamalpet cotton fields as being typically infested with *Fusarium vasinfectum*-wilt for a period of years tends the author to consider the isolate in question to be a strain of *Fusarium vasinfectum*, although more data are needed to confirm this.

Experiment I.—300 gm. each of air-dry sieved compost soil were taken in 6 containers. The 6 containers consisted of the following treatments, with 3 jars for each treatment :

(1) Soil unsterilised,

(2) Soil sterilised.

The Cholodny's slides were incubated in the moist chambers for 3 days before burial. 2 slides were buried in each jar. The 2 slides from one jar of each treatment were taken out after incubation for 7, 14 and 28 days. The results have been incorporated in Table I.

Table I clearly brings out the effect of microbial antagonism on *Fusarium vasinfectum* mycelium buried in the unsterilised soil.

* Detailed work on the mechanical and chemical analyses of these soils is in progress, and the relation of the same to the behaviour of *Fusarium vasinfectum* will form the subject of a subsequent communication.

TABLE I

Results of experiment I showing behaviour of Fusarium vasinfectum *in sterilised and unsterilised soil after* 7, 14 and 28 *days*

No.	Treatments	Attacked, decomposed, or healthy	Vegetative mycelium			Conidia and Chlamydospores	Remarks
			Incubation period				
			7 days	14 days	28 days		
1	Soil unsterilised	Attacked and decomposed	Attacked, decomposition gone half-way through	Attacked, decomposition almost complete	Absent, decomposition complete	No conidia or chlamydospores were seen (presumably because none were formed either before or after burial of the slides)	Besides bacteria, 3 or 4 other filamentous fungi could be seen
2	Soil sterilised	Healthy, not attacked or decomposed	Abundant	Abundant	Abundant	Present; increased in number with the period of incubation of the slide in the soil	Chlamydospores intercalary or terminal, thick-walled without ornamentations

Progressive decomposition of the mycelium was seen in the weekly observations until ultimately no trace of hyphæ could be recognized. 28 days' incubation represented complete decomposition of all mycelia of *Fusarium vasinfectum* in the unsterilised soil. In the sterilised soil none of these changes could be noticed. Indeed, the fungus produced conidia and chlamydospores prolifically due to the absence of competition for the available food material by the soil-inhabiting micro-organisms and also due to the survival of the mycelium without the decomposing effect of the soil organisms so clearly seen in the unsterilised soil.

Experiment II.—Both the soils mentioned above were used in this experiment. 16 container jars were used, with 2 slides in each jar. The slides were incubated in the soil for 4 days in the case of unsterilised treatments and for 15 days in the case of sterilised treatments. A summary of the various treatments is given below :

Series	*Sub-series*	*Treatments*
I. Compost Soil	(*a*) Unsterilised	1. Untreated (control)
		2. + 0·3% Ca $(OH)_2$
		3. + Calcium phosphate (monobasic) 1·0%
		4. + stable manure 3·0%
	(*b*) Sterilised	5. Untreated (control)
		6. + 0·3% Ca $(OH)_2$
		7. + Calcium phosphate (monobasic) 1·0%
		8. + stable manure 3·0%
II. Udamalpet Soil	(*a*) Unsterilised	9. Untreated (control)
		10. + 0·3% Ca $(OH)_2$
		11. + Calcium phosphate (monobasic) 1 0%
		12. + stable manure 3·0%
	(*b*) Sterilised	13. Untreated (control)
		14. + 0·3% Ca $(OH)_2$
		15. + Calcium phosphate (monobasic) 1·0%
		16. + stable manure 3·0%

The results obtained are shown in Table II.

Table II presents the following results : (*a*) in the unsterilised soils of both types (*i.e.*, Compost Soil and Udamalpet Soil) in all the different treatments, *F. vasinfectum* mycelium was attacked and decomposed by other soil organisms, more rapidly where stable manure had been added. Calcium phosphate (monobasic) retarded to some extent the rate of decomposition of the mycelia ; (*b*) in the sterilised soil, the growth of *F. vasinfectum* was quite healthy, and was considerably accelerated with profuse spore-forming tendencies when stable manure or phosphate was added.

TABLE II

Results of experiment II showing the effect of various soil amendments on the behaviour of Fusarium vasinfectum *in sterilised and unsterilised soil*

Series	Sub-series	Treatments	Whether attacked and decomposed or healthy	Stage of decomposition *	Vegetative mycelium	Conidia	Chlamydospores	Growth on soil surface and sides of container	Growth on slide surface	Remarks
I Compost Soil	*a.* Unsterilised	1. Untreated	Attacked	++	Attacked	None could be seen, evidently decomposed	Present, but attacked by bacteria	None	None	
		2. +0·3% $Ca(OH)_2$	do	+++	do	Surrounded and attacked by bacteria	Attacked by bacteria and fungi	do	do	Various stages of decomposition of conidia and chlamydospores by bacteria and of chlamydospores by certain unidentified filamentous fungus. Final digestion of the chlamydospores by the filamentous fungus
		3. Calcium monobasic phosphate 1·0%	do	+	do	Numerous	Numerous	do	do	Decomposition slower than in other treatments of the series

		4. +3·0% stable manure	do	++++	Decompos completely	None, evidently decomposed	Fairly numerous, surrounded and attacked by bacteria	do	do	Decomposition fastest among treatments of series. Slides especially characterised by the presence of certain mycelial forms certain rounded unicellular forms. Micro-flora more varied and dense than in other treatments of the series
	b. Sterilised	5. Untreated	Healthy, not attacked or decomposed	..	Abundant	Present many	Present many	None on soil suface, fine meshes of mycelia on sides of vessel	Fairly good growth	
		6. +0·3% $Ca(OH)_2$	do		do	Present	Present	Very little	Not much	
		7. +Calcium phosphate (monobasic) 1·0%	do		do	Present, numerous	Present, numerous	Better growth on soil surface and sides of vessel than in (5) or (6)	do	Conidia more numerous than chlamydospores
		8. 3·0% stable manure	do		do	do	do	Maximum fluffy white growth on soil surface, mycelium had penetrated to bottom of vessel	Least fluffy growth	Conidia and chlamydospores more numerous than in "7"

TABLE II—(*Contd.*)

Series	Sub-series	Treatments	Whether attacked and decomposed or healthy	Stage of decomposition*	Vegetative mycelium	Conidia	Chlamydospores	Growth on soil surface and sides of container	Growth on slide surface	Remarks
II Udamalpet Soil	*a.* Unsterilised	9. Untreated	Attacked and decomposed	++	Attacked	Present, surrounded and attacked by bacteria	Present, surrounded and attacked by bacteria	None	None	
		10. +0·3% $Ca(OH)_2$	do	++	do	None, evidently decomposed	Present	do	do	
		11. +Calcium monobasic phosphate 1·0%	do	+	Attacked, but much of mycelium intact	Present, numerous	Present, numerous	do	do	Extent of decomposition much less than in (9) or (10)
		12. +3% stable manure	do	++++	Attacked and decomposed	None, already decomposed	Present	do	do	Maximum decomposition
	b. Sterilised	13. Untreated	Healthy, not attacked or decomposed	..	Abundant	Present, numerous	Present, numerous	Mycelial ramifications on sides of vessel only, no growth on surface	More fluffy than when burried in soil	

		14. +0·3% $Ca(OH)_2$	Healthy, not attacked	..	do	Present	Present	None	Less growth than in (13) or (16) below	
		15. +Calcium monobasic phosphate 1·0%	do	..	do	Numerous	Numerous	Mycelial ramifications on sides of vessel only, no growth on soil surface	Same as in (14)	
		16. +3·0% stable manure	do	..	do	do	do	do	Some fluffy growth	Chlamydospores more numerous than in (13)

* Stages of decomposition :—

\+ Decomposition started.

\++ Decomposition gone half way through ; undecomposed filaments still surrounded by bacteria.

\+++ Decomposition almost complete.

\++++ Decomposition complete, no trace of *Fusarium* mycelium.

Discussion

Microbiological antagonism as an important factor affecting the pathogenicity of various fungi is now well recognized. Excellent reviews on the subject have been published by Garrett (1934, 1939, 1944); Garrard and Lochhead (1938); Porter and Carter (1938); D'aeth (1939); Weindling (1938); Waksman (1941), and many others. With the realization of the importance of the biotic factor of the soil in relation to the incidence of soil-borne diseases and their control, the explanation for certain very old agricultural practices became quite obvious. The principle underlying all such practices consisted mostly in enhancing the general microbiological activity of the soil by cultural practices like manuring, etc. Much useful work along these lines was carried out by many investigators during the past 20 years all of which points undoubtedly to the fact that in the case of soil-borne diseases, disease incidence due to certain soil-borne pathogens is inversely proportional to the antagonistic micro-floral content of the soil. Manuring was one of the methods by which such increased microbial activity was achieved for the control of many soil-borne diseases. It has been established that the role manuring plays in the control of these diseases is primarily in its relation to increased microbial numbers, although it has not been proved that the role of manuring in plant disease control is not related to increased host resistance.

It is evident from the present study that the microbiological factor of the soil is equally important from the point of view of eliminating dangerous soil-borne pathogens during their saprophytic phase. For instance it has been found during this investigation that the saprophytic activity of *Fusarium vasinfectum* Atk., is very limited in the bare soil. In fact the fungus makes no spread at all in the soil. It is, on the other hand, parasitized upon by antagonistic bacteria until finally it is completely decomposed. Thus, it is found that the life of *Fusarium vasinfectum* in the soil is similar to that of *Ophiobolus graminis* (Garrett, 1936) in that it alternates between a parasitic ascendant phase in the presence of the host and a saprophytic descendant phase in the absence of the host. Regarding the latter phase of the fungus in the soil, on the basis of the data presented in this paper, *Fusarium vasinfectum*, the fungus causing Cotton Wilt, is provisionally placed in Garrett's (1944) class of fungi making no extensive spread through the soil.

It is considered that the deterioration and quick disappearance of *Fusarium vasinfectum* in unsterilised soil, far from being a case of the chemical, physical or even nutritional unsuitability of the soil, is, on the other hand, similar to that of *Ophiobolus graminis* (Sanford and Broadfoot, 1931), and is attributable to the rapid elimination of the pathogen from the soil due to the operation of the microbiological factor.

It has been shown further that dormant stages of *Fusarium vasinfectum*, *e.g.*, conidia and chlamydospores, can also be parasitized upon by antagonistic micro-organisms, both fungi and bacteria. This points to the possibility of biological control of Cotton Wilt by elimination of the dormant resting stages of the causal fungus in the fallow soil.

As regards the relative persistence of the mycelial and dormant stages of *Fusarium vasinfectum* in the soil, it has been shown that the chlamydospores persist for a longer time in the soil than the vegetative mycelium of the fungus or the conidia. In some treatments, *viz.*, I (*a*) 4 and II (*a*) 12 (see Table II), even though no trace of the mycelium or the conidia could be seen, chlamydospores were still present. This indicates that chlamydospores are more resistant to decomposition than the vegetative mycelium or the conidia.

The response of *Fusarium vasinfectum* in the sterilised soil to both manure and phosphate was somewhat similar : manuring accelerated the growth of the fungus, whereas phosphate was found to enhance the production of conidia and chlamydospores. However, it was found that in the unsterilised soil addition of manure accelerated decomposition, whereas phosphate retarded it. The retardation of decomposition of the *Fusarium* mycelium in the phosphate treatment was, therefore, a result primarily of the direct effect of the phosphate on the accelerating effect it had on the growth of the pathogen and presumably not on the general microflora. The acceleration of decomposition obtained with addition of manure was due to the increase in the activity of the soil organisms already present in the soil as well as the further increase in the activity produced by the addition of a complex flora contained in the manure.

SUMMARY

A study of some factors especially microbial antagonism on the growth and survival of the cotton wilt fungus, *Fusarium vasinfectum* Atk., has been made.

In the unsterilised soils the introduced pathogen was in all cases attacked and decomposed by the antagonistic soil microflora, especially bacteria.

Decomposition in all cases was usually found to have been completed in 1–4 weeks, varying according to conditions.

In the sterilised soil in all cases the introduced pathogen showed healthy growth and was characterised by the production of numerous conidia, mostly micro-, and also large number of intercalary and terminal chlamydospores.

The effect of certain soil amendments on the antagonism of microorganisms to *Fusarium vasinfectum* was studied. While 0·3% calcium hydroxide did not have any appreciable effect, 3% manure accelerated the decomposition of the *Fusarium* mycelium by the antagonistic microflora, and 1·0% monobasic phosphate of calcium retarded decomposition to some extent.

The effect of the same treatments on *Fusarium vasinfectum* in sterilised soil was also studied. It was found that 3·0% manure increased the vegetative activity of the fungus to a great extent and 1·0% calcium monobasic phosphate enhanced the production of chlamydospores and conidia by *Fusarium vasinfectum*. Calcium hydroxide was not found to have any appreciable effect on the vegetative or reproductive activity of the introduced pathogen.

The author wishes to express his great indebtedness to Dr. T. S. Sadasivan, M.Sc., Ph.D. (Lond.), Director, University Botanical Research Laboratory, Madras, for suggesting the problem and for his constant guidance and help throughout the course of this investigation and in the preparation of this paper. He is grateful to Prof. M. O. P. Iyengar for going through the manuscript and offering suggestions.

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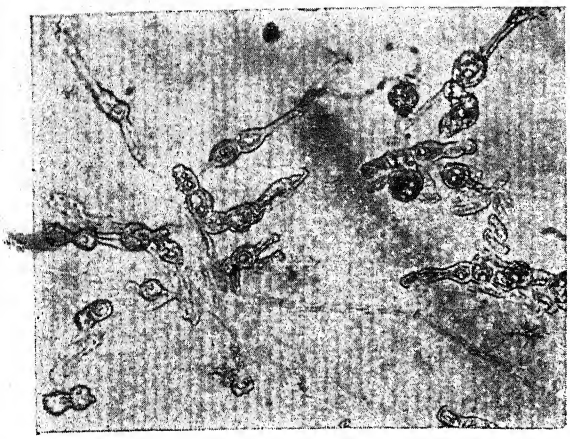

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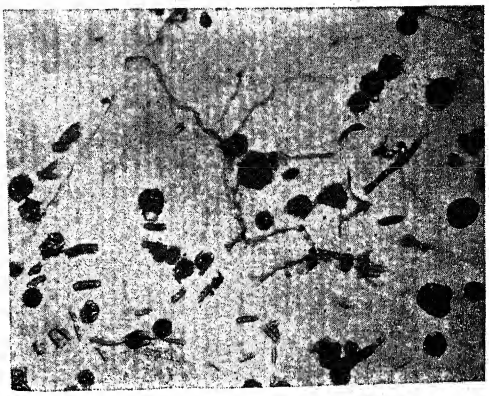

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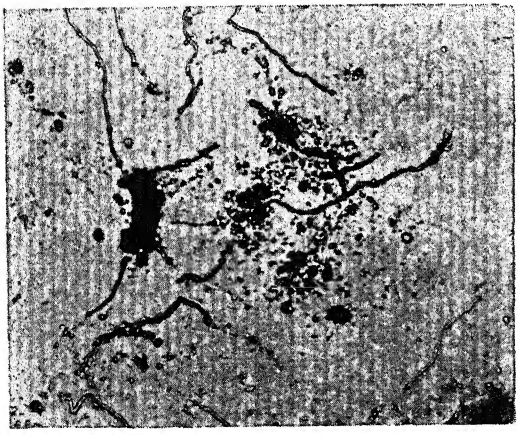

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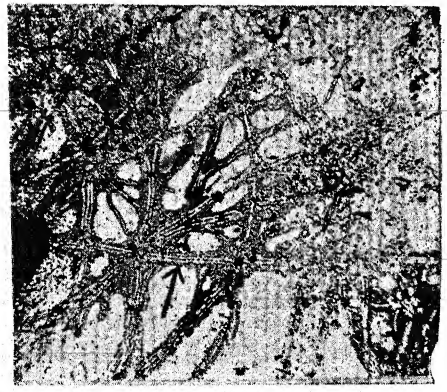

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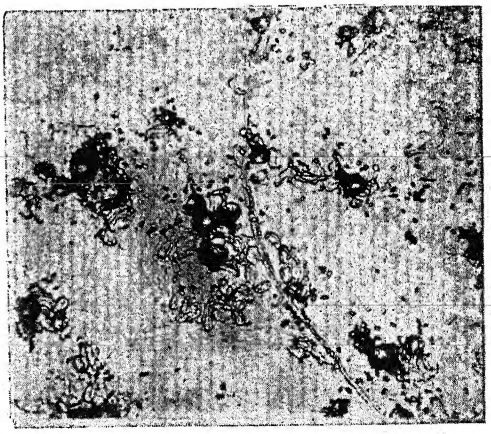

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C. V. SUBRAMANIAN—

EXPLANATION OF THE PLATE

Figs. 1 & 2. Behaviour of *Fusarium vasinfectum* in sterilised soil. Note unattacked healthy mycelium with many conidia and chlamydospores. Fig. 1 from Expt. II, treatment 16 ; Fig. 2 from Expt. I, treatment 2. ×400.

Figs. 3–6. Behaviour of the fungus in unsterilised soil.

Figs. 3–5. Successive stages in the decomposition of the *F. vasinfectum* mycelium by antagonistic bacteria.

Fig. 3. Initial stage of decomposition. From Expt. I, treatment 1. ×336.

Fig. 4. Intermediate stage of decomposition. From Expt. II, treatment 10. ×104.

Fig. 5. Final stage of decomposition. Also from Expt. II, treatment 10. Arrow points to vacant space originally occupied by the *Fusarium* mycelia. Note the dense bacterial growth surrounding the vacant space. ×104.

Fig. 6. Conidia and chlamydospores of *F. vasinfectum* surrounded by bacteria. From Expt. II, treatment 2. ×344.

A NOTE ON THE INFLORESCENCE OF *RICINUS COMMUNIS* LINN.

By S. N. Chandrasekaran and D. Daniel Sundararaj

Agricultural College and Research Institute, Coimbatore

Received for publication on February 15, 1946

Though *Ricinus communis* Linn. has been generally recognised to be monœcious, there has been some confusion as to the position of the male and female flowers on the main axis of the inflorescence. In some standard works, such as *Genera Plantarum* by Bentham and Hooker and the *Flora of British India* by Hooker, the female flowers are described to be arising at the bottom and the male flowers towards the top of the inflorescence, whereas Jussieu in his *Genera Plantarum* mentions that the male flowers are found towards the base and the females towards the top. These, in addition to the note in *Science and Culture* of April 1945, made us examine and study thoroughly the inflorescence of *Ricinus communis* from plants cultivated and growing wild as escapes at Coimbatore.

The inflorescence is an erect terminal branched raceme of cymes with staminate flowers at the lower portion of the flowering axis and the pistillate flowers towards the top. The lower branches of the racemes generally bear male flowers, though sometimes they have some female flowers towards the top. There are also rare cases where these two types of flowers are indiscriminately mixed and borne on the main axis of the inflorescence. W. B. Joshi (*Poona Agric. Coll. Mag.*, 1926, **18**, 20–22) records occasional cases of the "existence of dicœcious plants" and plants with hermaphrodite flowers.

As early as 1768, in a drawing of *Ricinus speciosus* Burm., a synonym of *Ricinus communis* Linn. by Burmanni in *Flora Indica*, one pistillate flower is shown below with a number of male flowers above in the inflorescence. But the figures in Rheede's *Hortus Malabaricus* and *Rumphius Amboinense*, though not quite representative and true as the drawing in Curtis' *Botanical Magazine*, clearly bring out the inferior position of the male flowers and the superior position of the females on the inflorescence. In 1877, Kurz in his *Flora of British Burma* describes *Ricinus* as having female flowers in the lower part of the flowering axis. This mistake appears to have crept in Bentham and Hooker's *Genera Plantarum* of 1880 and has been repeated in several later publications. Prain in Thiselton Dyer's *Flora of Tropical Africa* and *Flora Capensis* follows Bentham and Hooker for the generic description of *Ricinus*; but for *Ricinus communis* Linn., he rightly describes "the males below and females higher up". This mistake is also seen in the floral descriptions in the following publications, and requires the necessary corrections : (1) *The Botany of Bihar and Orissa* and (2) *Forest Flora of Chota Nagpur* by Haines. (3) *Flo a of Aden*, *Rec. Bot. Surv. Ind.* (4) *Flora of Madras* by Gamble. (5) *Indian Medicinal Plants* by Kiritikar and Basu. (6) *Cyclopedia of American Horticulture* by Bailey.

PALMOXYLON SCLERODERMUM SAHNI FROM THE EOCENE BEDS OF NAWARGAON, WARDHA DISTRICT, C.P.

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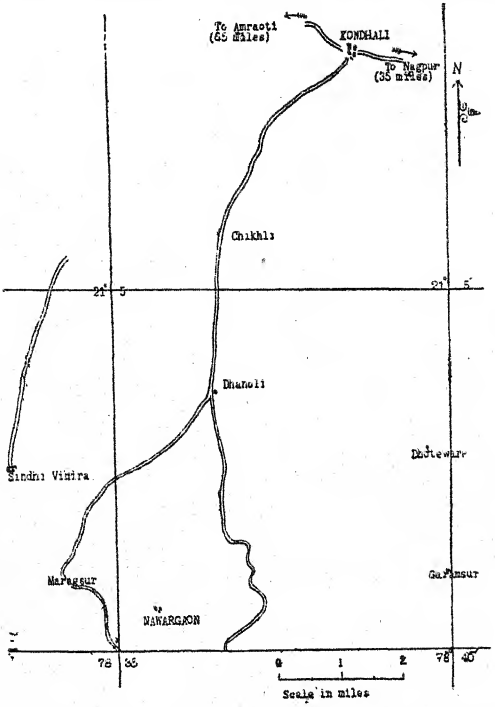
Introduction

A few years ago the author came across a petrified palm stump at the Central Museum, Nagpur. This stump had been collected by Mr. K. P. Sagreiya, I.F.S., in the forest area of Nawargaon (Lat. 21° 1′, Long. 78° 35′), Wardha district, C.P. (Text-fig. 1), in the year 1934 along with another palm stump and several fragments of petrified dicotyledonous woods. The entire collection was presented by Mr. Sagreiya to the museum. Suspecting this specimen to be a new



Text-Fig. 1

species of *Palmoxylon*, the author borrowed it through the kindness of the Curator for investigation. During the course of study the author compared it with the numerous species of petrified palms in the collection of Professor B. Sahni, F.R.S., which includes at least 45 new species of Indian Palmoxyla. It was discovered that the present species was already represented in that collection and had been named by Professor Sahni as *Palmoxylon sclerodermum* sp. nov. (Sahni, 1943). As the Nagpur specimen is more complete than the type specimen, the author on the advice of Professor Sahni undertook the re-investigation of the species based on the present specimen, in which the entire girth of the stem is preserved, with the roots in organic connection.

I am very much indebted to Professor Sahni who very kindly placed at my disposal his entire collection of palms for comparison and also his MS account of the different species. I may also take this opportunity of expressing my heartfelt thanks to my Professor for his kind guidance, valuable suggestions and criticism during the progress of this work.

The fact that fossil plants have been found in the region of Nawargaon has been recorded by Haines (1916, p. 5) and later by Sagreiya (1936, p. 2), but so far as I am aware no account of any petrified plants from this locality has yet been published. The fossils occur within an area of about four square miles and are best exposed along the slopes of a valley to the east of a cultivated field in Maragsur. They are embedded in lateritic morrum and can be easily excavated. Sometimes they lie partially or entirely exposed. Like most of the other beds in the Nagpur-Wardha region, these beds may also in all probability be referred to the Base of the Deccan Intertrappean Series, the age of which is now believed to be Eocene (Sahni, 1934).

Pieces of fossil wood also occur in the adjoining cultivated fields and in the region of Nawargaon forest village in the Hingni range.

Description

This solitary specimen is a brown coloured stump 1′–4″ long and 1′ in diameter at the base, narrowing to 8″ at the top (Photo. 1). Numerous roots are present at the base in organic connection forming a thick mantle round the stem (Photo. 15); their preservation is as good as that of the main stem. In the upper part of the stem the sheath of roots is replaced by a cortical zone about 3 cm. thick which surrounds the whole stem. It is possible that part of this cortical zone is really formed by the decurrent bases of leaves, but no persistent leaf-bases can be distinguished in the fossil.

Before proceeding with the description of the specimen, it may be advisable to say a few words about the descriptive terms used in the present paper. I have found it convenient to follow in the main the descriptive terminology used by Professor Sahni in his manuscript on the Indian petrified palms which I have consulted, and also in his account of the type specimen of the present species (Sahni, 1943).

Inwards the cortex, three zones may be distinguished in the stem: the dermal, subdermal and central (Photo. 5, D, S.d., N.). This

division into three zones is based on the following scheme adopted by Prof. Sahni :—

Bundle distribution and form of sclerenchyma	*Zone*	*Orientation of fibro-vascular bundles*
Bundles crowded, *sclerenchyma* deformed by contact	Dermal	Usually normal
Bundles not crowded ; *sclerenchyma* retains its normal form	Subdermal	Usually normal
	Central	Irregular

Similarly, the term *f/v* has been used to indicate the ratio between the cross-section area occupied by the sclerenchyma and by the vascular part of a fibrovascular bundle. I have also adopted the descriptive terms the dorsal and the ventral *sclerenchyma*, median sinus, auricular sinus, auricular lobes, etc., for the parts of the fibro-vascular bundle.

The *cortex* is about 3 cm. in width (Photo. 2, C.).

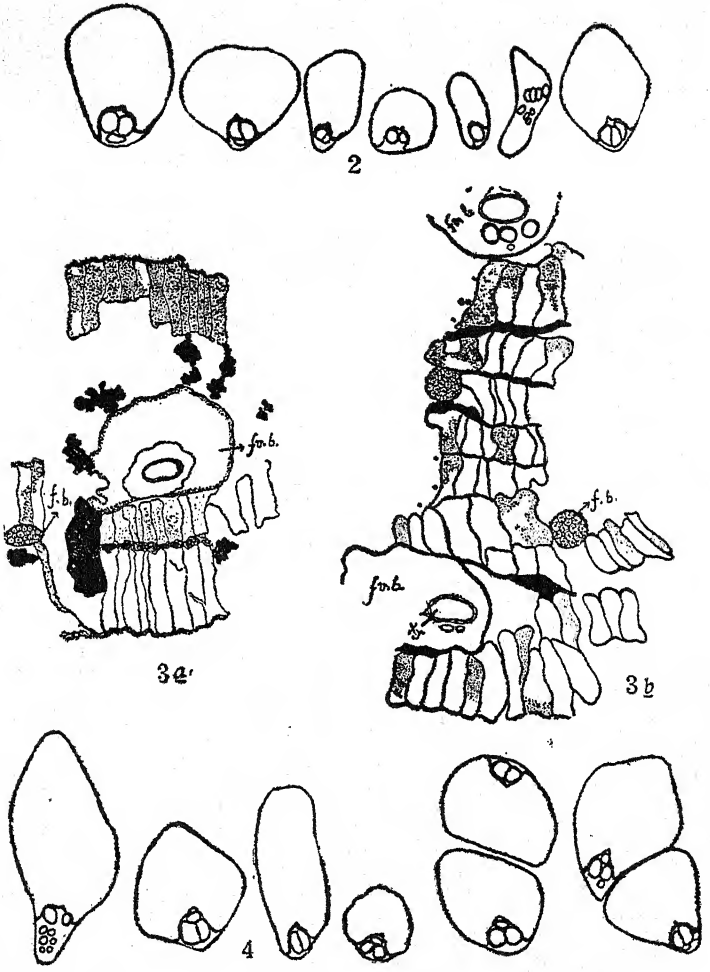
The fibrovascular bundles in this region as seen in cross-section are of varying form and size (Photo. 3, Text-fig. 2). Their average diameter across the *sclerenchyma* is ·35 mm. As a rule they are oval in shape, with a pointed end. The median sinus is cordate and xylem usually contains 3 to 4 vessels. The *f/v* ratio varies from 4/1 to 6/1. The *fibrous bundles* are scattered in the ground tissue and are usually of even size. A single bundle may on an average be made up of 12 to 15 fibres. Stegmata are present both on the fibrous and fibro-vascular bundles.

The lacunar ground tissue is made up of parenchymatous cells which are light-brown coloured and mostly radially elongated and rectangular. They are usually arranged parallel to each other and often occur in layers (Photo. 3, Text-fig. 3).

The *dermal region* is about 2·5 cm. thick. The fibrovascular bundles, on an average 105 per cm.^{2} and all normally orientated, are usually elliptic and as a rule larger than those in the cortex.

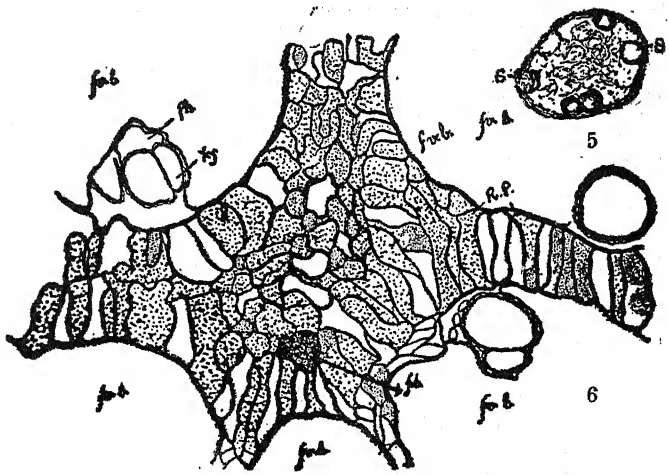
These bundles are again of varying form and size (Text-fig. 4). The smaller bundles being about ·4 mm. in diameter, the larger ones about twice as thick. These bundles are usually pressed against each other and show one or more flat sides. The auricular lobes are mostly rounded. In the xylem there is usually a single large median vessel and the *f/v* ratio varies from 9/1 to 18/1. The phloem (always very badly preserved) lies deep in the angles of cordate sinus. The fibrous bundles occur only here and there and are very much like those of the leaf-base region.

Stegmata are constantly present on the fibrous bundles (Text-fig. 5) and also on the fibrovascular bundles. The ground tissue is lacunar and composed of thin walled isodiamatric cells. It occurs in the form of tiny patches between the fibrovascular bundles which are very compact and at places where the bundles are very close to each other it may be only a single cell in thickness (Photo. 4).



Text-Figs. 2–4. Fig. 2. Some fibrovascular bundles from the cortical zone. × 50. Figs. 3 *a* and *b*. Two areas from the cortex showing radially elongated cells; *f.b.*, fibrous bundle; *fv.b.*, fibrovascular bundle; *Xy*, xylem. × 102. Fig. 4. Some fibrovascular bundles from the dermal zone. × 50.

A few leaf-trace bundles also occur in this region. They are distributed radially at great intervals throughout the zone and being cut obliquely appear radially stretched (Photo. 4, 1t.). The dorsal *sclerenchyma* forms the major portion in these bundles but the xylem too is quite prominent. The vascular part comprises a number of vessels of medium size and the tongue-like process forms well-defined acute angled auricular sinuses with the auricular lobes. There also occurs a patch of ventral *sclerenchyma* at the extremity of this tongue-like process. The parenchymatous cells present over the projecting process are usually radially stretched.



Text-Figs. 5–6. Fig. 5. A fibrous bundle as seen in cross-section from the dermal zone showing stegmata (S) round it. × 205. Fig. 6. A patch of ground tissue from the subdermal zone as seen in transverse section. *fv.b.*, fibrovascular bundle ; *Xy.*, xylem ; *Ph.*, Phloem ; *f.b.*, fibrous bundle. × 102.

The *subdermal zone* is nearly 2·5 cm. in width. The fibrovascular bundles (about 75 per cm.^{2}) are usually quite separate from each other and thus retain their normal form (Photo. 6) so that the form and structure of the bundles can be satisfactorily studied. They are mostly normally orientated and their average diameter as seen in cross-section is 1 mm. Their general outline is circular or slightly elliptic. The dorsal margin of the sclerenchyma is quite round and its base is cordate. Tabular parenchyma is often present in one or two layers (Photo. 10, T.). Auricular sinuses are quite insignificant as the auricular lobes merge insensibly into the sides of the xylem. Median sinus is quite well marked in most of the bundles. *Phloem* which is wedged in this sinus is often very poorly preserved. *Xylem* is usually 3 or 4 vessels, the larger two occurring ventrally and the other smaller ones placed internally to them (Photo. 6). Pitting of the metaxylem vessels is either scalariform or, more commonly, reticulate (Photo. 14, R.). The end walls of the vessels are very oblique and have scalariform thickening with wide spaces between the bars (Photo. 12, W.). *Fibrous bundles* occur sporadically in the ground tissue, a single bundle being usually made of 12–18 fibres. *Stegmata* are present both on the fibrous and the fibrovascular bundles and are best seen in longitudinal sections. Wherever the *sclerenchyma* is cut radially, the stegmata appear in two longitudinal rows along the margins (Photo. 11, S.) but if it is cut tangentially, they are seen to occur in several rows (Photo. 13, S.).

The *ground tissue* (Text-fig. 6) which here occupies an area comparatively larger than in the dermal region, is lacunar and made up of compact, rather isodiametric, small, thin-walled cells which appear slightly lobed in transverse section (Photo. 9, Text-fig. 6). At the

Comparison with the type specimen of P. sclerodermum *Sahni.*

As the central region is not present in the type specimen, the comparison is necessarily limited to the outer zones only.

The cortex of both the specimens is similar in having both fibrous and fibrovascular bundles of different sizes with stegmata round them. It is, however, not possible to compare the ground tissue, as the type specimen includes only a narrow zone (6 mm.) of this region and the tissue is very badly preserved there.

Next, coming to the dermal zone it is seen that the frequency of the fibrovascular bundles is almost identical in this specimen (108 per cm.^{2} in the type specimen and 105 in the present one). Their form, size and average diameter are also very similar and so is the fibrovascular ratio which varies only between 12/1–18/1. The occurrence of stegmata both on the fibrous and fibrovascular bundles, the presence of a lacunar ground tissue which is made of compact thin-walled isodiametric cells, and the characteristic form of the leaf-trace are again features which indicate a close affinity between the two.

Extending our comparison to the subdermal zone, we again find that the fibrovascular bundles are of a similar size (1 mm. in both) but there is a slight difference in their frequency : about 65 per cm.^{2} in the type specimen and 75 in the corresponding region of our fossil. This variation, however, may be considered negligible as the frequency of the bundles may easily vary within the same zone along different radii, even in one and the same tree. Similarly, the slight variation observed in the *f*/*v* ratio (18/1–25/1 in the type specimen and 18/1–22/1 in the Nagpur specimen) need not be attached any great importance. The median sinus in both the specimens is cordate and stegmata are present both on the fibrous and the fibrovascular bundles.

The greatest resemblance is seen in the ground tissue which in both the specimens is lacunar and made up of thin-walled, rather isodiametric and lobed cells, with idioblasts scattered among them here and there. In my specimen I have described some palisade cells in the ground tissue of the subdermal zone. These are not recorded in the type specimen, but as stated, that specimen includes only a very small peripheral portion of the subdermal zone. The form and structure of the leaf-traces is essentially similar in the two specimens.

The central zone, as stated above, is not present in the type specimen, but from the details available in the specimen here described it is observed that there is hardly any difference between the subdermal zones of either of the two specimens and the central zone available in this material except for the irregular orientation and smaller frequency of the fibrovascular bundles.

The conclusion is therefore fully justified that the specimen here described is specifically identical with *P. sclerodermum* Sahni.

Palmoxylon sclerodermum-Sahni

(Plates VI-IX ; Text-figs. 2–8)

Diagnosis : Cortex—Cells of the ground tissue in the cortex radially elongated and arranged in tiers, fibrovascular bundles scattered, fibrous bundles present. Stegmata on both the fibrous and the fibrovascular bundles.

Dermal zone—Fibrovascular bundles in the dermal region flattened against each other and distorted, normally orientated, nearly 105 per cm.,^{2} ·4 to 1 mm. in diameter, xylem usually consists of a single median vessel, phloem very badly preserved and wedged in the cordate median sinus, *f*/*v* ratio 9/1–18/1 : fibrous bundles present, stegmata both on the fibrous and fibrovascular bundles : ground tissue lacunar, compressed in between the fibrovascular bundles, made of isodiametric thin-walled cells, leaf-traces tangentially cut and appear radially stretched with well-developed xylem projecting as a tongue-like process.

Subdermal zone—Fibrovascular bundles of the subdermal zone not compressed, round or elliptic, nearly 85 per cm.,^{2} mostly normally orientated, about 1 mm. in diameter, *f*/*v* ratio nearly 20/1, auricular lobes rounded or at times merge into the sides of the xylem elements, base of the dorsal sclerenchyma distinctly cordate, in xylem usually two large median vessels placed side by side. Phloem very badly preserved ; fibrous bundles present ; stegmata present both on the fibrous and fibrovascular bundles. Ground tissue lacunar, compact, of isodiametric slightly lobed cells ; palisade cells also occur at places. Leaf-traces as in the dermal zone.

Central zone—Fibrovascular bundles in the central zone quite free, nearly 75 per cm.^{2}, usually round, irregularly orientated, about 1 mm. in diameter, in other respects very like those of the subdermal ; fibrous bundles present ; stegmata both on the fibrous and fibrovascular bundles. Ground tissue very like in the subdermal, slightly larger. Leaf-traces absent.

Roots—4–8 mm. in diameter, outer cortex sclerenchymatous, inner made of compact cells at the periphery but very much lacunar on the inner side, 20–24 bundles in the stele, each bundle made of 3–4 vessels. Phloem (badly preserved) in the form of elongated patches between the xylem bundles, pith sclerenchymatous.

Localities—(1) Type specimen (coll. Burton), Seoni, Chhindwara district, (2) The present specimen (coll. Sagreiya) Nawargaon, Wardha.

Horizon—Base of the Deccan Intertrappean Series (Eocene). Reg. No. F/275 (present specimen).

Affinities

Now, as the anatomy of the complete specimen is known, the present species on the basis of the median cordate sinus of the dorsal *sclerenchyma* might be referred to the group *Cordata* of the Corypha-like palms in Stenzel's classification (1904, pp. 149–51). But by studying only some of the outstanding features of three known species under this group, it can be concluded that the present species is entirely different from any of them.

Comparison with Cordata *group.*

P. Fladungi Unger and *P. angulare* Cotta differ from the present species in having a much protruding vascular part of the fibrovascular bundle and in the absence of fibrous bundles. *P. geanthraci* (Göppert) Stenzel differs in having an ovate fibrous part in the fibrovascular bundles and numerous fibrous bundles.

Plate VII. Photo. 5. Entire transverse section through the upper part of the stump; C, cortex ; D, dermal ; Sd., subdermal ; N, Central zones (×6/7).

Plate VIII. Photo. 6. Transverse section through the subdermal region (×25).

Photo. 7. A patch of ground tissue from the subdermal zone ; R, palisade cells in the ground tissue (×70).

Photo. 8. Part of transverse section through the central zone showing irregular orientation of fibrovascular bundles (×25).

Photo. 9. Part of the ground tissue from the central region showing slightly lobed cells ; F, a fibrous bundle (×140).

Photo. 10. A typical fibrovascular bundle with its surrounding ground-tissue from the central region as seen in transverse section ; T, tabular parenchyma at the periphery of the bundle (×50).

Plate IX. Photo. 11. Longitudinal section showing S, a row of stegmata with dorsal sclerenchyma to its left and parenchyma to the right ; (×140).

Photo. 12. Longitudinal section showing a vessel with its scalariform end wall (W) (×140).

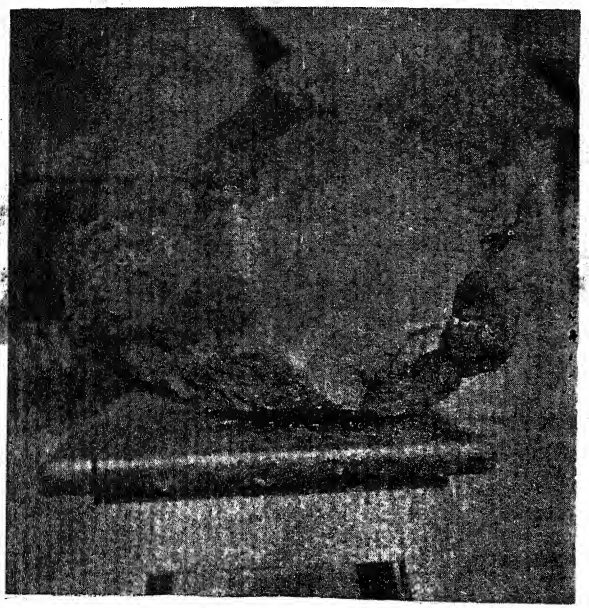
Photo. 13. Longitudinal section through the stem of *P. sclerodermum* Sahni showing S, rows of stegmata on the dorsal sclerenchyma cut tangentially with parenchyma on the right (×140).

Photo. 14. Longitudinal section showing a vessel with reticulate thickening (R) (×210).

Photo. 15. Basal part of *P. sclerodermum* Sahni ; R, roots fractured transversely (×1/4).

Photo. 16. Transverse section through a group of roots seen in Photo. 16 (×3/2).

Photo. 17. Transverse section through the root, O.C., outer cortex ; I.C., inner cortex ; 1, peripheral part of the inner cortex ; 2, central part of the inner cortex ; A, lacunar spaces ; L, phloem ; P, pith. (×25).



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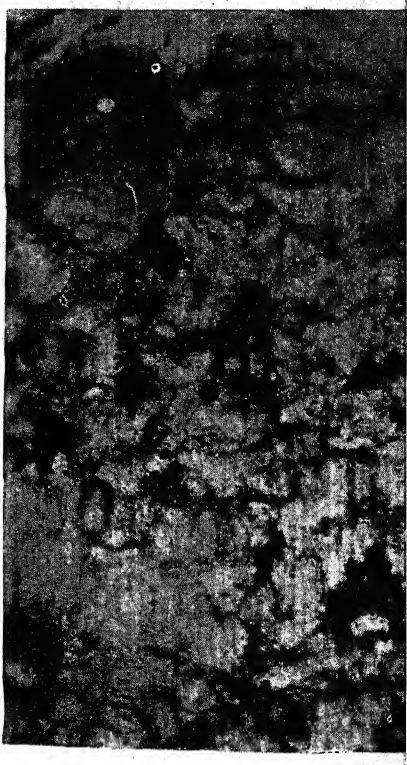

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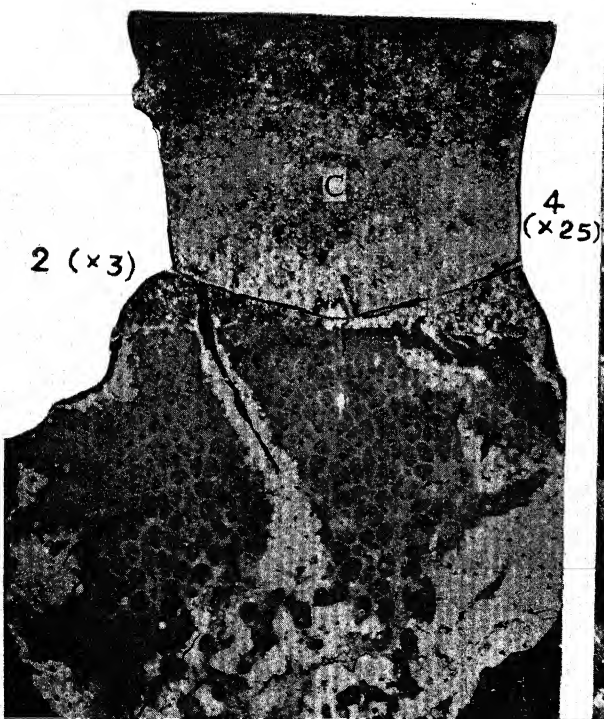

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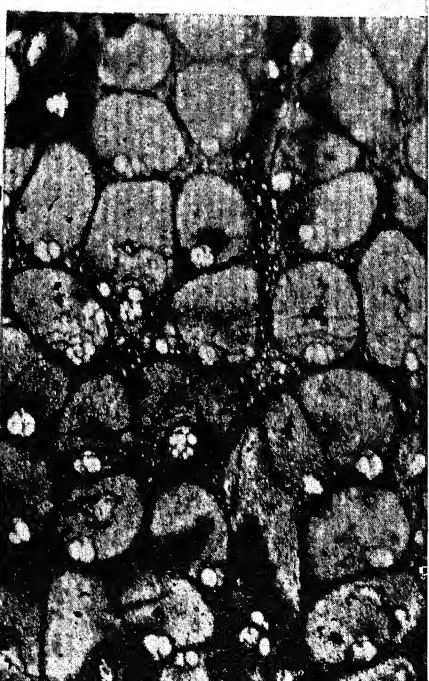

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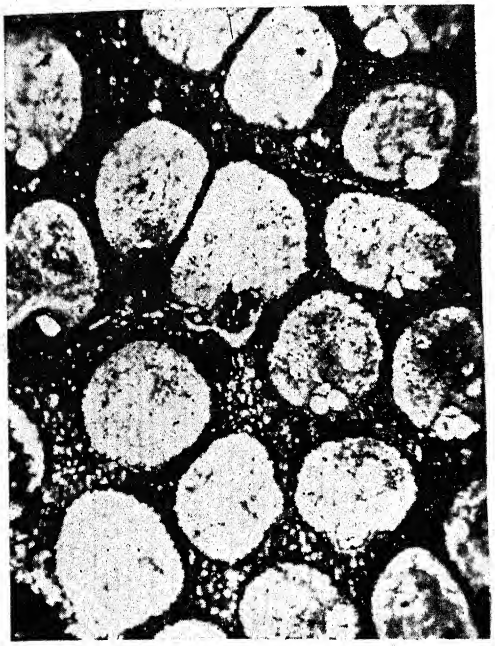
V. B. SHUKLA, PHOTOS—

PALMOXYLON SCLERODERMUM *SAHNI FROM THE EOCENE BEDS OF NAWARGAON, WARDHA DISTRICT, C.P.*



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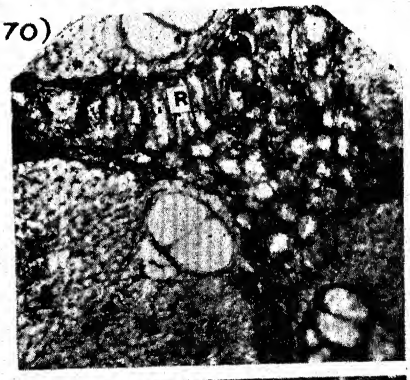

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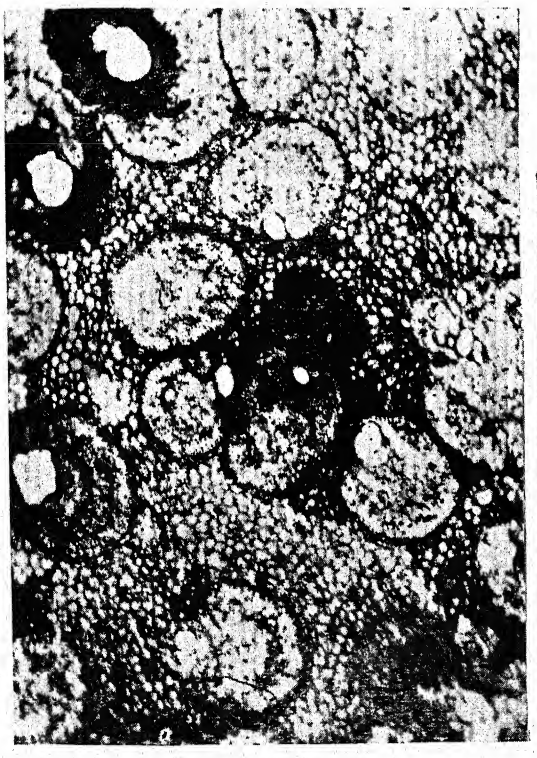

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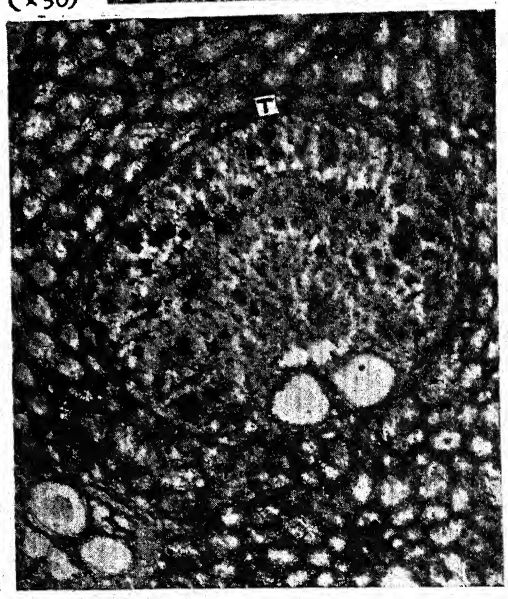

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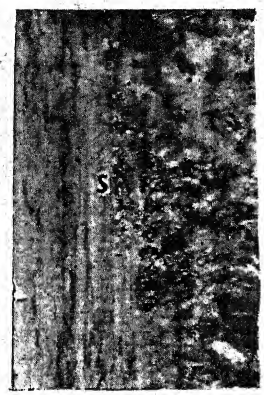
V. B. SHUKLA, PHOTOS—

PALMOXYLON SCLERODERMUM *SAHNI FROM THE EOCENE BEDS OF NAWARGAON, WARDHA DISTRICT, C.P.*



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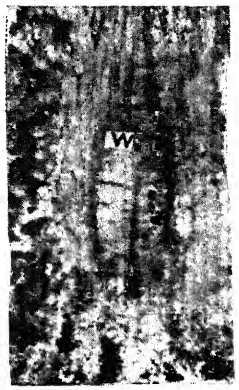

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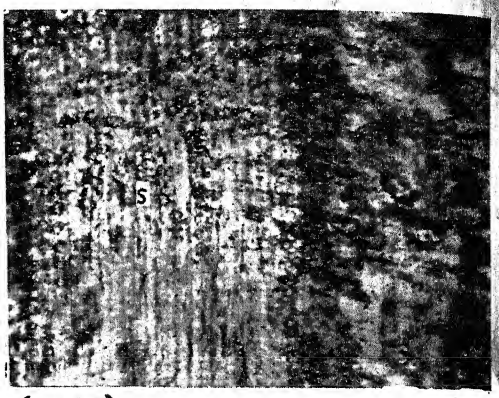

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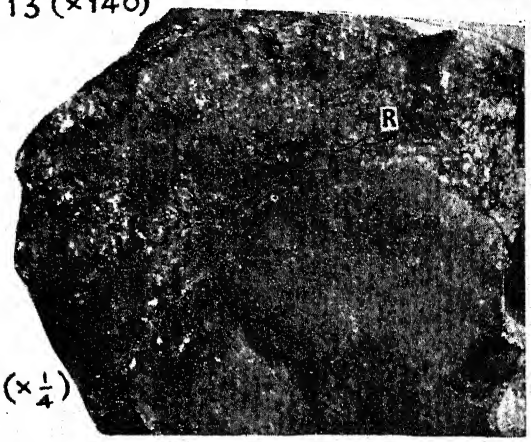

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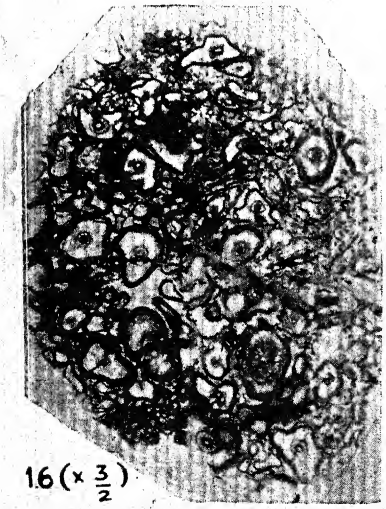

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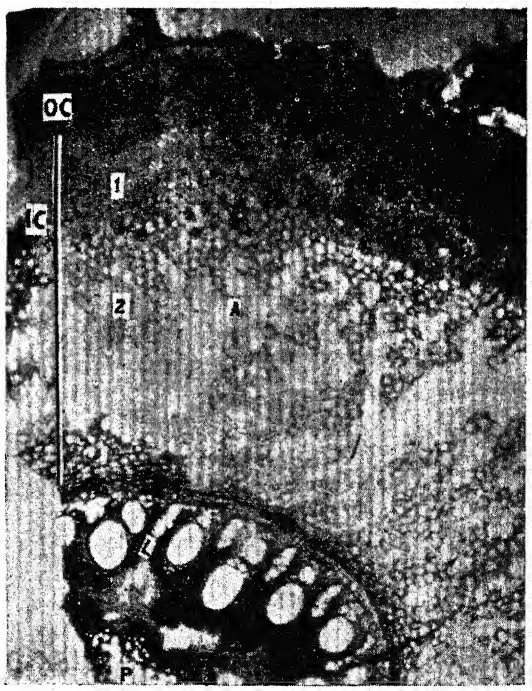

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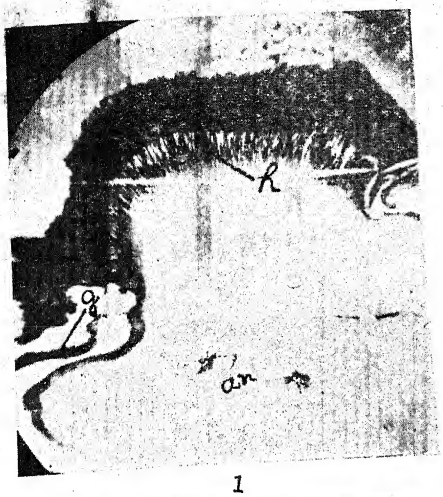
V. B. SHUKLA, PHOTOS—

PALMOXYLON SCLERODERMUM *SAHNI FROM THE EOCENE BEDS OF NAWARGAON, WARDHA DISTRICT, C.P.*



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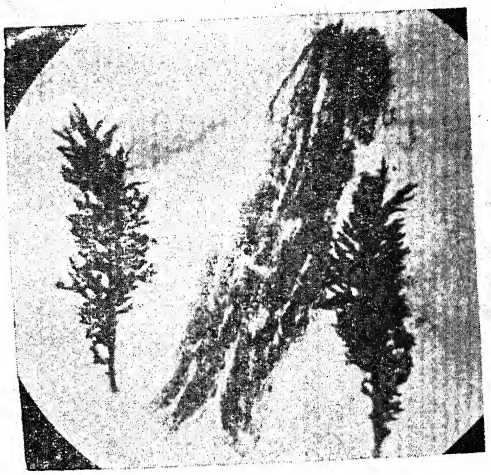

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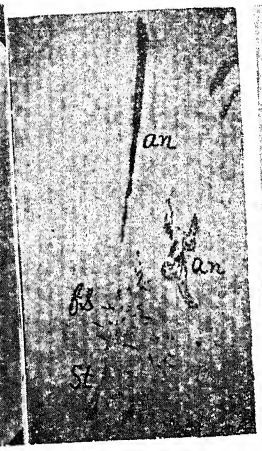

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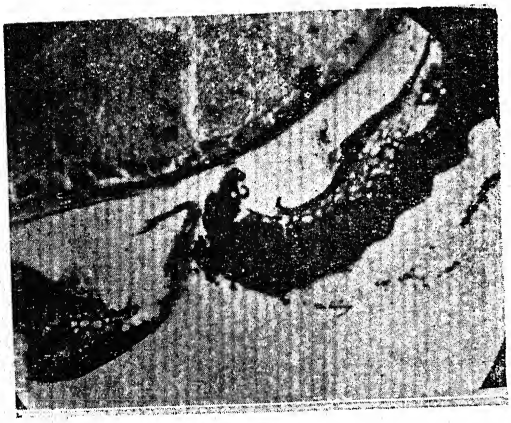

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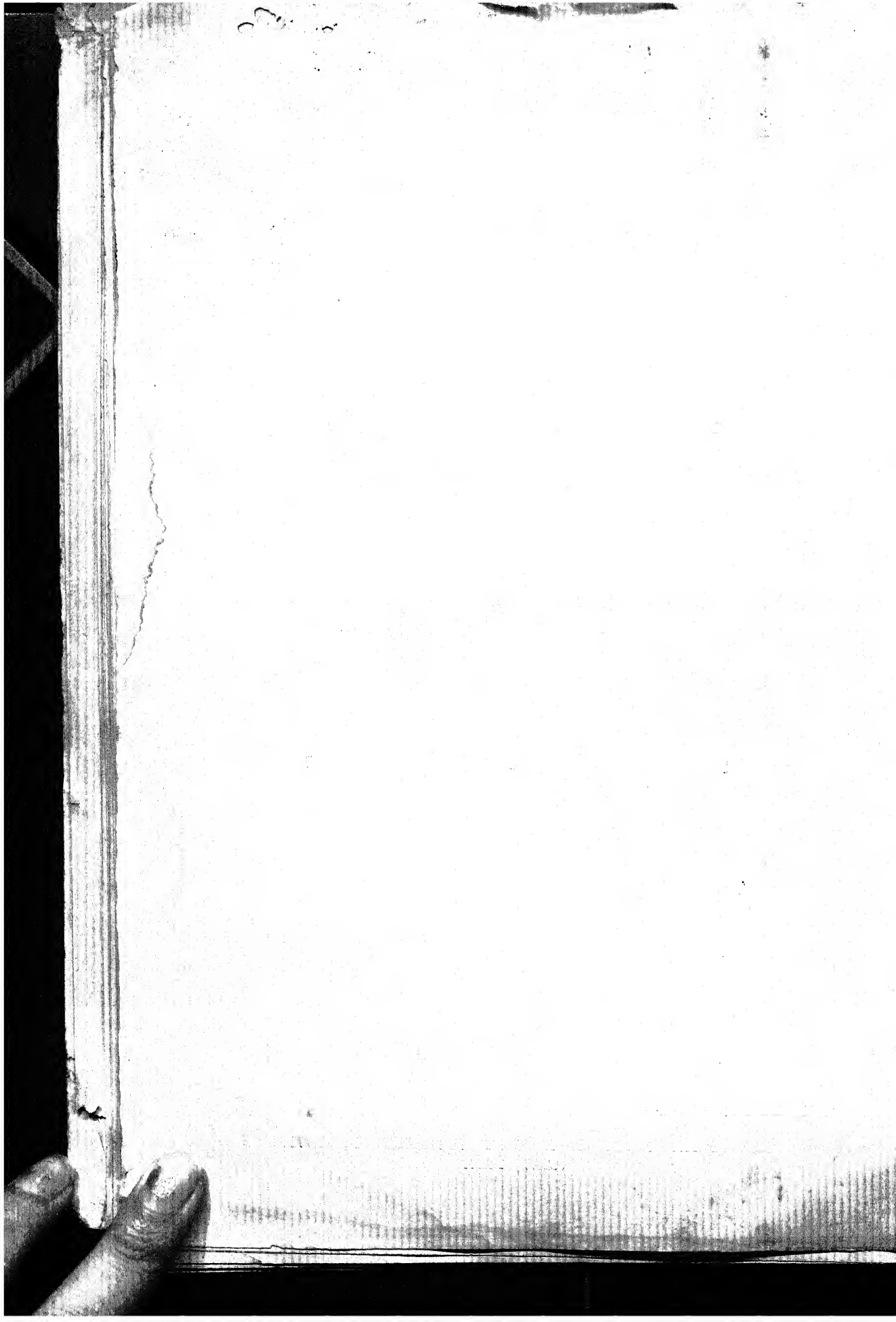
S. P. RAYCHAUDHURI—

MODE OF INFECTION OF RICE BY USTILAGINOIDEA VIRENS *(CKE.) TAK.*



ON *JOHANNESBAPTISTIA PELLUCIDA* (DICKIE) TAYLOR AND DROUET FROM MADRAS

By M. O. P. Iyengar and T. V. Desikachary

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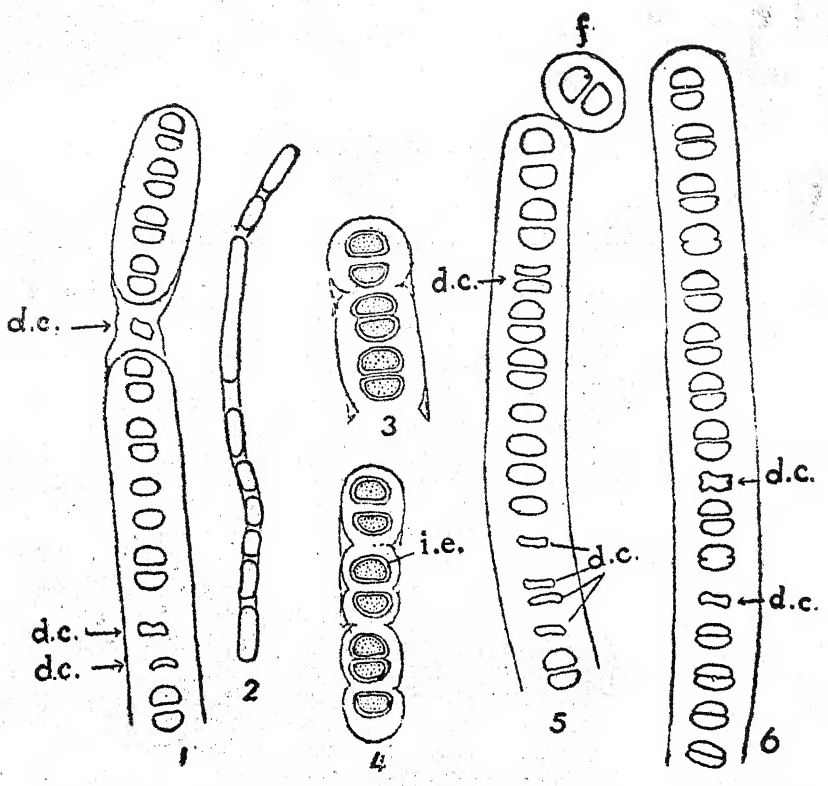
In 1927, Gardner recorded from Porto Rico two blue-green algæ which he referred to a new genus, *Cyanothrix* (non *Cyanothrix* Schmidle), and called the two algæ *C. primaria* and *C. Willei.* Taylor described in 1928 an alga from Dry Tortugas under the name *Nodularia* (?) *fusca* sp. nov. Frémy (1935, p. 96) with regard to this new species says that Taylor evidently had no knowledge at the time of the establishment of Gardner's *Cyanothrix*, otherwise, he (Taylor) would have doubtless made it a *Cyanothrix* intermediate in dimensions between *C. primaria* and *C. Willei.* De Toni, in 1934, renamed Gardner's genus *Cyanothrix* as *Johannesbaptistia* (Gardner) De Toni, since the name *Cyanothrix* had already been given by Schmidle (1897, also 1898) for an alga (*Cyanothrix vaginata*), which is now included under the genus *Mastigocladus* Cohn. Frémy (1935) found in some algal collections from the Isle of Bonaire and Algeria a *Johannesbaptistia* which showed characteristics of all the three species, *viz., J. primaria* (Gardner) De Toni, *J. Willei* (Gardner) De Toni and *Nodularia* (?) *fusca* Taylor, and so combined all these three under one species, *J. Gardneri* Frémy comb. nov. Two years later Seurat and Frémy (1937, p. 294) recorded this alga from South Tunisia also.

Drouet, in 1936, made a detailed study of a collection of *Johannesbaptistia* from the Galapagos Islands and also the original materials of Gardner and Taylor. He agreed with Frémy's combination of all the known species under one species, but preferred to use the name *J. primaria* (Gardn.) De Toni instead of *J. Gardneri* Frémy for nomenclatural considerations. Taylor (Drouet, 1938) from an examination of authentic material of *Hormospora pellucida* Dickie (Dickie, 1874) came to the conclusion that *H. pellucida* was really a *Johannesbaptistia.* Taylor and Drouet (Drouet, 1938), therefore called the alga *Johannesbaptistia pellucida* (Dickie) Taylor and Drouet and made *J. primaria* (Gardn.) De Toni (*J. Gardneri* Frémy) a synonym of it.

The writers found a *Johannesbaptistia* in a collection of algæ from a brackishwater pool at Ennore, a place about 10 miles north of Madras. This genus does not appear to have been recorded so far from India. A detailed account of the alga is given here.

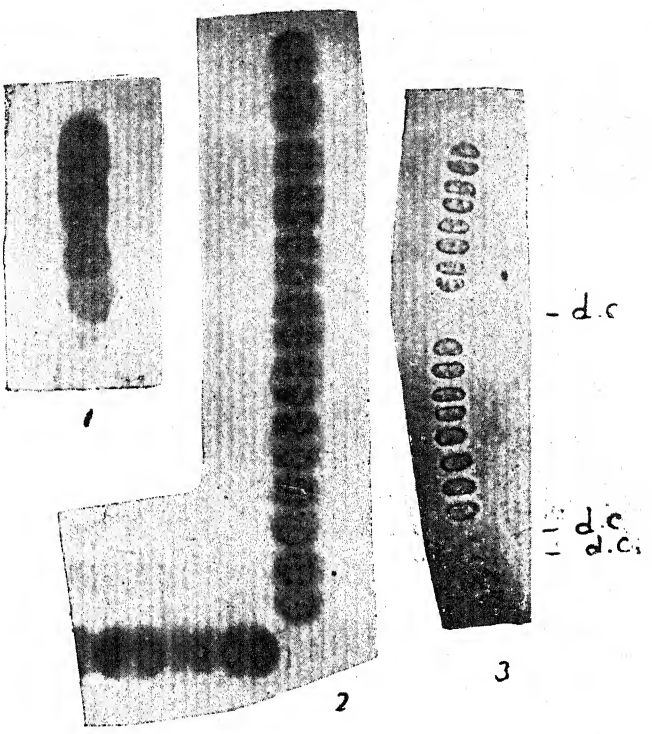
The alga is filamentous and has a broad mucilaginous sheath. The sheath is hyaline and not refractive. Occasionally its outer limits



Text-Figs. 1-6. *Johannesbaptistia pellucida* (Dickie) Taylor and Drouet.

Fig. 1. Portion of a filament. Note the fragmentation near a dead cell. Same filament as in Plate X, Fig. 3. Fig. 2. A long filament which is breaking up into a number of fragments drawn under low power. Cells not shown. Fig. 3. Portion of a filament drawn after staining with safranin showing the common mucilage as well as the individual envelopes. Fig. 4. A short filament stained with safranin showing the common mucilage as well as the individual envelopes. Same as in Plate X, Fig. 1. Figs. 5 & 6. Portions of filaments ; in Text-fig. 5 a two-celled fragment, just separating from the end portion. (*d.c.*, dead cell ; *i.e.*, individual envelope ; *f.*, fragment.). Text-figs. 1, 3–6, × 1165 ; Text-fig. 2, × 300.

This alga in the writers' opinion clearly belongs to the Chroococcales and is a filamentous development among the Chroococcales. Such a filamentous condition can easily be derived from a Chroococcaceous condition by the limiting of the cell-division to one plane only. The filamentous condition of *Johannesbaptistia* must be considered as merely a parallel development among the Chroococcales. A filamentous tendency is already seen in some of the members of the Entophysalidaceæ (see Fritsch, 1945, p. 819 ; Geitler, 1932, p. 293). The genus *Johannesbaptistia* may be considered as the highest expression of the filamentous tendency among the Chroococcales. The writers, therefore, agree with Gardner (1927), Geitler (1932) and Drouet (1936) that *Johannesbaptistia* should be kept in the Chroococcales. They



Figs. 1 and 2. Filaments stained with safranin showing the mucilaginous envelope round individual cells and also round pairs of daughter cells.

Fig. 3. An unstained filament showing fragmentation; note dead cells (*d. c.*) at the region of fragmentation.

All × 850.

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JOHANNESBAPTISTIA PELLUCIDA (*DICKIE*) *TAYLOR AND DROUET FROM MADRAS*

therefore retain the original diagnosis of the genus *Johannesbaptistia* (Gardn.) De Toni and are unable to accept the emended diagnosis of the genus of Frémy (1935, p. 99). They suggest that the genus may be placed in the Entophysalidaceæ along with other algæ which show a filamentous tendency.

Summary

An account is given of *Johannesbaptistia pellucida* (Dickie) Taylor and Drouet. The alga was collected at Ennore near Madras. This appears to be the first record of this genus in India.

The writers do not agree with Frémy's suggestion that the genus *Johannesbaptistia* should be placed in the Oscillatoriaceæ between *Oscillatoria* and *Lyngbya*, but agree with Drouet's suggestion that it should be retained inside the Chroococcales.

The filamentous condition in *Johannesbaptistia* should be considered as a parallel development among the Chroococcales. *Johannesbaptistia* represents the highest expression of the filamentous tendency among the Chroococcales.

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SOME STUDIES ON THE SMUT, *USTILAGO COICIS* BREF., OF JOB'S TEARS MILLET

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Received for publication on January 22, 1946

I. Introduction

Job's tears millet (*Coix lachryma-jobi* L.) is an important cereal in the Central Provinces, Sikkim, Assam and Burma and the plant is grown as a regular field crop. The plant is very hardy in nature and thrives upon almost any kind of soil, yielding a good amount of produce. In taste, the cereal resembles wheat. In Assam the plant is grown in Khasi, Garo and Naga Hills. The grain is roasted, then husked and eaten whole, either parched or boiled. The grain is also milled and ground to flour and baked into bread. It is also used as a poultry feed and in the Naga Hills utilized for the manufacture of a beer called *dzu* which is highly prized for its fruity flavour and delicate aroma.

The plant is susceptible to a smut, *Ustilago coicis* Bref., in the Khasi Hills and is affected every year. The disease is widespread and causes considerable damage. Estimates made have revealed that the damage caused usually amounts from 12 to 25 per cent. and in extreme cases the loss amounts to more than 35 per cent. Every grain of the head is transformed into a black spore mass, without much increase in size as compared with the healthy grains. The spore mass is surrounded by a membrane hidden by the glumes and is traversed by flattened or angular filaments probably the remains of the fibro-vascular bundles of the axis. The fungus is ovaricolous and completely destroys the ovaries, all of which in a raceme are destroyed. Plate XI shows the symptoms of the disease.

Mundkur (1941) has reported a second smooth-spored smut on this millet which he calls *U. lachryma-jobi* Mundkur from Girnar Hills but this smut has not so far been observed to occur in Assam.

II. Morphology of Spores

The sori are 9–13 mm. long and 5–9 mm. broad, brown-black in colour and contain a pulverulent spore mass. Spores are held together by the hard floral glumes. The spores (Fig. 1) are liver-brown, subglobose to ellipsoidal with minute but clear echinulations which give the margin a serrate appearance ; rather prominent circular pits are also present on the epispore. The spores are 7–13 μ (mean 9·1 μ) in diameter.

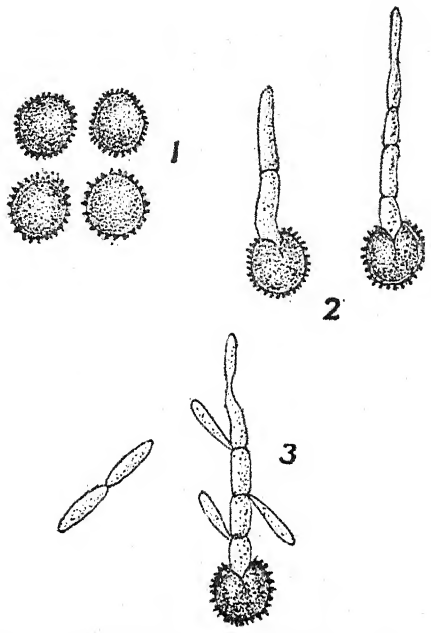

Fig. 1. Spores. Fig. 2. Germination of spores with formation of promycelium.
Fig. 3. Germination of spores with formation of sporidia.

III. Germination of Spores

The spores germinate freely in water or nutrient solutions as soon as mature. The spore on germination gives rise to a promycelium which is always four-celled (Fig. 2). Sporidia (Fig. 3) are formed terminally and laterally near the septa and these may bud off secondary sporidia. The primary sporidia often elongate into a septate filament which may be longer than the promycelium and which also buds off secondary sporidia from the ends and from near the septa.

(i) *Effect of nutrient solutions on germination.*—The spores were germinated at 25°–26° C. in glucose and sucrose solutions, in Job's tears leaf-juice, soil extract, dung extract and distilled water. Job's tears leaf-juice was prepared by steaming for half an hour 20 grams of green leaf in 100 c.c. of distilled water ; soil extract was obtained by dissolving 10 grams of soil in 100 c.c. of distilled water allowing the solution to stand for 24 hours and then filtering through ordinary filter paper ; cowdung extract was prepared by shaking 10 grams of it in 100 c.c. of distilled water, allowing it to stand for 24 hours and then filtering through ordinary filter paper. The results obtained are recorded in Table I.

It will appear from the data presented in Table I that the nutrient solutions exerted a favourable influence on spore germination and the percentage of germination was more in all the nutrient solutions than

TABLE I

Effect of nutrient solutions on spore germination

Media	Percentage of germination after	
	24 hours	48 hours
2% glucose solution ..	17	49
5% ,, ,, ..	29	79
2% sucrose ,, ..	16	52
5% ,, ,, ..	31	81
Job's tears leaf-juice ..	28	65
Soil extract ..	27	67
Cow-dung extract ..	29	62
Distilled water ..	12	27

in distilled water. Five per cent. glucose and five per cent. sucrose solutions were found better than 2 per cent. solutions of these sugars. Job's tears leaf-juice, soil extract and cow-dung extract were almost equally effective, the difference being very little though inferior to 5 per cent. sugar solutions.

(ii) *Effect of temperature on germination.*—The spores were germinated in 5 per cent. glucose solutions at temperatures ranging from 5° to 40° C. The results obtained are recorded in Table II.

TABLE II

Germination of smut spores at different temperatures

Temperature (C.)	Percentage of germination after	
	24 hours	48 hours
5	..	..
15	14·9	56·4
20	16·2	71·4
25	29·8	89·2
30	45·8	94·0
35	23·7	45·8
40	..	..

The results presented in Table II show that the optimum temperature for the germination of spores is about 30° C., the maximum between 35° and 40° C. and the minimum between 5° and 15° C.

(iii) *Effect of hydrogen-ion concentration on spore germination.*—The spores were germinated at 25° C. in 5 per cent. glucose solution having a hydrogen-ion concentration range varying from 3·0 to 9·0. Normal hydrochloric acid and normal sodium hydroxide solutions

were used to make different pH ranges and the hydrogen-ion concentration was determined by the calorimetric method. The observations are recorded in Table III.

TABLE III

Germination of smut spores at different pH values

Hydrogen-ion concentration	Percentage of germination after	
	24 hours	48 hours
3·0	30·0	82·1
3·8	31·2	80·0
4·2	29·0	79·2
5·2	35·2	88·7
6·0	46·9	91·7
6·4	52·8	97·5
7·0	43·4	89·2
7·7	40·2	87·7
8·4	32·8	82·8
9·0	27·8	74·2

From the results recorded in Table III it is clear that the germination of spores is very good over a wide pH range. The optimum hydrogen-ion concentration for spore germination, however, is 6·4.

IV. MODE OF TRANSMISSION OF THE DISEASE

The following pot and field experiments were carried out to study the mode of perpetuation of the disease.

(i) *Experiments to test seedling infection by seed-borne spores.* In 1943 the soil and pots used for the experiments were sterilized in an autoclave at 120° C. for two hours. Forty such pots were sown on 20th April with Job's tears seed which had previously been steeped in a 2 per cent. solution of formalin for 20 minutes, then dried and smeared with spores of the smut by shaking them in a glass vessel containing a thin paste made of spores in distilled water. Ten other pots were sown on the same day with Job's tears seeds similarly steeped in formalin solution and dried but not smeared with spores, to serve as controls. All the plants received upto the time of harvesting the same cultural treatment. On the 7th October the number of healthy and smutted plants was as given in Table IV, from which it will be seen that while the controls were free from smut, 25 per cent. of the seeds infected with smut spores gave smutted plants. The above experiment was repeated in 1944 with similar results, which are also contained in Table IV.

Another similar experiment was carried out in a small plot but the soil was not sterilized. It was known however that Job's tears millet was never grown before on this land or round about this field for a distance of about 50 miles. Seeds infected in the manner of the

TABLE IV

Summary of results of infection experiments carried out in pots

Year	Control				Infected seed			
	Total plants	Healthy plants	Smutted plants	Per cent. smut	Total plants	Healthy plants	Smutted plants	Per cent. smut
1943 ..	40	40	Nil	Nil	160	120	40	25·0
1944 ..	40	40	,,	,,	120	93	27	20·9

last experiments were sown in 7 rows on 15th April and 7 rows were sown with seeds treated as for the controls mentioned above, cultural treatments being kept the same for both. On the 5th October 29·6 to 35·7 per cent. of the plants were smutted in the infected rows while none of the others had smut.

TABLE V

Summary of field infection studies carried out with infected seeds

Rows of plants	Control				Infected seed			
	Total plants	Healthy plants	Smutted plants	Per cent. smutt	Total plants	Healthy plants	Smutted plants	Per cent. smut
1	102	102	Nil	Nil	98	63	35	35·7
2	96	96	,,	,,	108	76	32	29·6
3	64	64	,,	,,	96	66	30	31·2
4	82	82	,,	,,	112	72	40	35·7
5	112	112	,,	,,	127	85	42	33·1
6	75	75	,,	,,	116	81	35	30·1
7	107	107	,,	,,	99	67	32	32·3

The above experiment was repeated in 1944 with similar results. These results from pot and field experiments show that seed-borne spores are a source of infection in this smut.

(ii) *Experiments to test seedling infection by spores shed in the soil from the previous year's crop.*—On 27th October 1942 spores of smut from material collected on 7th October 1942 were mixed with the surface 2 to 3 inches of sterilized soil contained in 20 sterilized pots which were then placed in the open. On the 10th April 1943, disinfected Job's tears seeds were sown in them. Ten sterilized pots sown on the same day with disinfected seed but whose soil was infected with smut spores at sowing time, were arranged to serve as controls. The results recorded in October 1943 are presented in Table VI. Another similar experiment was carried out in a small plot but the soil was not sterilized. The plot was divided into two parts; on the 7th October 1942 spores of the fungus were mixed with the surface 2 to 3 inches of

the soil of one part of the plot and left fallow. On 10th April 1943 disinfected Job's tears seeds were sown on this and the other part of the plot, the soil of the latter being infected with smut spores immediately before sowing to serve as control. The results noted in October 1943 are also recorded in Table VI.

TABLE VI

Summary of soil infection experiments with smut spores

Treatments	Total plants	Healthy plants	Smutted plants	Per cent. smut
(*a*) *Pot Experiments*—				
Soil infected with spores at the time of sowing, April 1943 ..	40	28	12	30·0
Soil infected in October 1942 ..	80	80	Nil	Nil
(*b*) *Field Experiments*—				
Soil infected with spores at the time of sowing, April 1943 ..	329	234	95	28·8
Soil infected in October 1943 ..	346	344	2	0·58

These experiments were repeated in 1943–44 and exactly similar results obtained. These results show that the infection arising from spores carried in the soil from the previous year's crop is insignificant.

(iii) *Experiments to test floral infection.*—Fifty ears were bagged on fifty healthy plants of Jobs tears on 15th September 1943, thirty of which were dusted on three consecutive days, 20th, 21st and 22nd September 1943 with smut spores freshly collected each day. Each day after dusting a very little water was sprayed with an atomizer on the dusted ears which were then rebagged. The twenty other ears which were not dusted with spores were kept as controls.

Both the sets were unbagged on 20th October 1943 and the seeds collected from each kept separate. Each lot was then sown in two plots of land where Job's tears was never grown before. The seeds were disinfected before sowing in 2 per cent. formalin solution for 20 minutes ; sowing was done on 17th April 1944. The observations made are noted in Table VII.

TABLE VII

Summary of results of floral infection

Treatments	Total plants	Healthy plants	Smutted plants	Per cent. smut
Seed from infected flowers	387	387	Nil	Nil
Control ..	215	215	,,	,,

The results recorded in Table VII show that no infection comes from blossom infection.

From these experiments the conclusion arrived at is that the general mode of infection is seedling infection by seed-borne spores. When the seed germinates, the smut spores also germinate and the smut hyphæ penetrate the tissue of the seedling at once. The parasite keeps pace with the developing plant becoming conspicuous as black, sooty masses of spores which totally replace the flowering head. These studies further show that the chances of the disease being reproduced through soil-borne spores are remote and insignificant. This conclusion agrees with the finding of Thomas (1920), who first showed that this smut is seed-borne.

V. Control Measures

As the disease is primarily carried through infected seeds it is evident that to get a healthy crop it is necessary to sow only healthy seeds obtained from a disease-free locality or to disinfect the seeds, before sowing with such fungicides which will kill the smut spores adhering to the outside of the seeds without injuring the seeds. Four fungicides, namely, agrosan G, ceresan, formalin dust and copper carbonate dust were tested.

To test the efficacy of these fungicides an experiment was carried out at Sylhet in 1943. Naturally infected seeds were selected for treatments but they were given a further dose of artificial infection by shaking them in a glass vessel containing a thin paste made of smut spores in distilled water. The seeds were thereafter carefully shaken with the respective fungicides so that they got thoroughly and uniformly covered with the fungicides. The treatments were as follows :

(i) Control : Infected seeds.

(ii) Infected seeds treated with agrosan G at the rate of one part per 250 parts of seed by weight.

(iii) Infected seeds treated with ceresan at the rate of one part per 250 parts of seed by weight.

(iv) Infected seeds treated with formalin dust at the rate of one part per 200 parts of seed by weight.

(v) Infected seeds treated with copper carbonate dust at the rate of one part per 200 parts of seed by weight.

The treated seeds were sown immediately after treatments in April 1943. Randomised block system of lay-out was followed ; there were six replications. Each block contained five plots, each plot 10 ft. × 5 ft. in size. Each plot had twenty rows of plants and in each row there were twenty plants. At the time of harvest the number of smutted plants was carefully noted ; the percentage of smutted plants worked out is recorded in Table VIII.

It will be evident from the results presented in Table VIII that though none of the fungicides tried is able to control the disease completely, all of them are quite effective in holding the disease appreciably under check. In comparison with the percentage of smut in the control plots, the percentage of smut in the treated plots is insignificant. In order of efficacy the fungicides may be listed as follows : copper carbonate, formalin dust, agrosan G and ceresan.

TABLE VIII

Number and the percentage of smutted plants after seed treatments

Block	Control			Agrosan G			Ceresan			Formalin dust			Copper Carbonate		
	Total No. of plants	Smutted plants	Per cent. smut	Total No. of plants	No. of smutted plants	Per cent. smut	Total No. of plants	Smutted plants	Per cent. smutt	Total No. of plants	No. of smutted plants	Per cent. smut	Total No. of plants	No. of smutted plants	Per cent. smut
1 ..	400	130	32·5	400	6	1·5	400	8	2·0	400	3	0·75	400	1	0·25
2 ..	400	112	28·0	400	2	0·5	400	2	0·5	400	4	1·0	400	2	0·5
3 ..	400	121	30·25	400	2	0·5	400	2	0·5	400	2	0·5	400	0	0·0
4 ..	400	130	32·5	400	4	1·0	400	6	1·5	400	2	0·5	400	1	0·25
5 ..	400	108	27·0	400	3	0·75	400	3	0·75	400	3	0·75	400	2	0·5
6 ..	400	118	29·5	400	6	1·5	400	4	1·0	400	2	0·5	400	0	0·0
Mean	..	..	29·96	..	..	0·96	..	..	1·04	..	..	0·67	..	..	0·25

The experiments were repeated in 1944 and practically the same results obtained. Thus it can be safely concluded that the disease can be appreciably kept under control by treating the seeds with fungicides before sowing. To secure satisfactory results it is necessary that the seeds are thoroughly and uniformly covered by the fungicides.

VI. SUMMARY

Job's tears millet (*Coix lachryma-jobi*) is grown in Khasi, Garo and Naga Hills and is an exceedingly important cereal. The plant is susceptible to a serious smut due to *Ustilago coicis* in the Khasi Hills and occurs every year. The disease is wide spread and the damage caused usually amounts from 12 to 25 per cent. per annum.

The morphology of the spores has been described.

Influence of nutrient solutions, temperature and hydrogen-ion concentrations on the germination of the spores has been studied. The optimum temperature and hydrogen-ion concentration for spore germination is about 30° C. and 6·4 pH respectively.

The mode of transmission of the disease has been studied. It has been observed that the principal source of infection in this smut is the seed-borne spores.

Experiments on control measures consisted in treating the infected seeds before sowing with certain fungicides, namely, agrosan G, ceresan, formalin dust and copper carbonate dust. None of them was found able to completely control the disease but in comparison with the control plots the percentage of smut in the treated plots was insignificant ; copper carbonate dust was found to be the best.

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Symptoms of the disease

S. CHOWDHURY—

SOME STUDIES ON THE SMUT, USTILAGO COICIS *BREF., OF JOB'S TEARS MILLET*

A LEAF SPOT OF *BORASSUS FLABELLIFER* L. CAUSED BY *PESTALOTIA PALMARUM* CKE.

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Received for publication on January 22, 1946

I. Introduction

Til (*Borassus flabellifer*) is extensively grown in Assam for its fruit, the juice of which is used for the preparation of a special kind of delicious cake. The leaves are used for the manufacture of hand fans which command quite a wide and profitable market. The plant, however, suffers from a serious leaf spot caused by *Pestalotia palmarum* Cke., which kills almost the entire leaf and by thus destroying the food manufacturing apparatus of the plant makes it weak. A survey made during the past three years has revealed that in severe cases of leaf infestation the fruiting capacity of the plant is impaired. The disease also lowers very appreciably the value of the leaves as material for the manufacture of fans and in a great many cases makes them almost useless for the purpose.

The disease has been observed to occur throughout Assam; it has also been reported from Bombay by Uppal, Patel and Kamat (1935). Recently Mundkur and Kheswala (1942) have reported the occurrence of the parasite on *Areca catechu* L. in Chittagong, on *Borassus flabellifer* in Bengal and Bihar, on *Cocos nucifera* L. in Bengal and on *Phoenix sylvestris* Roxb. in Bombay and Bihar. In Assam, however, it has been observed so far only on *Borassus flabellifer*.

II. Symptoms of the Disease

In early stages of infection the disease is characterised by small, yellowish brown, circular to oblong, more or less depressed spots which are about a millimeter in diameter. The diseased areas gradually increase uniformly in length and width. The yellowish brown spots are seen surrounded by a grayish brown band which is about a millimeter in width. Sometimes this band does not extend completely around the spots. Within this brown band there is a cream-coloured portion which appears much thinner than the healthy parts of the leaf. In some cases the thinner central portion of the spots appear dark brown. The lesions tend to grow in length parallel to the veins.

In advanced stages of the disease the spots may be as large as five centimeters long and one or more centimeters wide. The central portion of the spot becomes gray. The brown band at the border becomes dark brown. Two to five or more spots may coalesce forming larger, irregular gray dead areas. On the upper surface of the leaf,

black minute dot-like bodies consisting of the fruiting structures of the fungus soon appear on the grayish center. Both young and old spots spread rapidly under damp humid conditions. If the air is very dry, the lesions develop very slowly and the spots do not develop until the leaves are old. Plate XII shows the symptoms of the disease.

III. Parasitism

Single spore culture of the fungus was obtained by the usual plating method and a series of inoculation experiments were carried out on *tál* plants that were about two to two and a half years' old. The leaves were rubbed gently for five minutes with absorbent cotton immersed in 1 : 1000 solution of mercuric chloride and then the disinfectant was washed off thoroughly with sterile distilled water. Inoculations were made as follows :

(i) By spraying the leaves on the upper and lower surfaces with a suspension of spores from pure culture.

(ii) By placing bits of culture containing the crushed black fruiting bodies of the fungus on the upper and lower surfaces of the leaves.

(iii) By placing bits of agar with the fungus on it on needle pricks made through the upper and lower surfaces of the leaves.

(iv) By spraying with a suspension of spores on needle pricks made through the upper and lower surfaces of the leaves.

On the third day infection was observed on all leaves inoculated through wounds, but no evidence of infection was noted on leaves inoculated without wounds even after 15 to 21 days. Young infections were characterised by yellowish brown spots which were about a millimeter in diameter. The spots were circular to oblong and somewhat shrunken. The lesions gradually increased in length and width. In advanced stages of infection the patches became gray, as in natural infection. The spots turned dark brown. The lesion soon coalesced forming larger irregular areas. The black fruiting bodies appeared on the gray centers of the lesions on the upper surface of the leaves in 7 to 11 days.

A large number of inoculations were made. In every case it was found that the fungus could infect only wounded surfaces of the leaves. From all the infected plants the original fungus was isolated in each case. Controls were kept and they remained healthy throughout the experiments.

Cross inoculation experiments were carried out and it was found that the fungus could infect only wounded leaves of *Areca catechu*, *Cocos nucifera* and *Phoenix sylvestris*.

IV. Morphology

Mycelium.—The mycelium of the fungus extends between the cells of the leaf, throughout the discoloured lesion on the leaf. The hyphæ are exceedingly fine, sparingly septate and colourless.

In pure culture both submerged and aerial hyphæ are produced. When young the submerged mycelium is densely granular, septate, hyaline, vacuolate and somewhat irregular in outline. The cross walls are somewhat hard to distinguish. When old, the granules of the mycelium disappear. The aerial mycelium, when young, is very sparsely granular and hyaline. When old granules seem to be absent from the cells of the mycelium.

Pycnidia.—Underneath the upper leaf epidermis, the hyphæ collect into masses which develop into bowl-shaped, thin walled spore cases, the pycnidia (Fig. 1), the basal wall of which is distinct, while the lateral and top walls are slender and rather obscure. In culture the pycnidia are present in large numbers on the loose mycelium growing on the medium and also on definite stroma composed of interwoven hyphæ. They are of diverse shapes and sizes and may be globose, spherical, rectangular or oval. The pycnidia formed on leaves measure 130 to 420 μ and those on oat agar range from 85 to 240 μ in diameter.

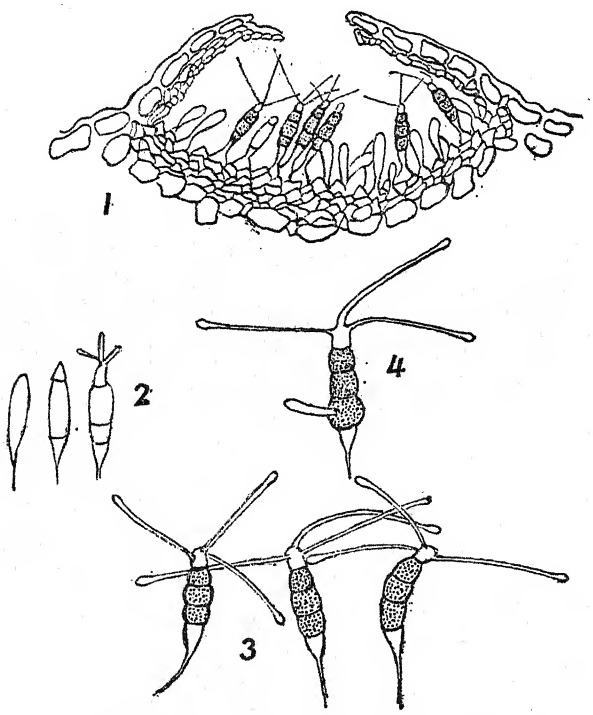


Fig. 1. Section through Pycnidium. Fig. 2. Stages in the development of conidia. Fig. 3. Mature conidia. Fig. 4. Germination of a conidium. Fig. 1, ×250; Figs. 2–4, ×450.

On the inner wall of the lower half of the pycnidium, a layer of spore-bearing cells occurs. From each of these, a stalk grows out into the cavity of the pycnidium and terminate in a spore. When

ripe, the spores are detached with the stalk, which remains as an appendage at the base of the spore. At the same time, the epidermis and the slender top wall of the pycnidium underlying it become raised up and then ruptured by pressure from below, opening outwards in a crack of irregular shape, through which the spores are liberated in such quantities that they collect in little black crusts round the mouth of the pycnidium. Spores from the pycnidia developed in culture are liberated by a rupture of the wall at the top, or sides to the exterior or at the base into the stroma or culture medium.

Conidia.—The conidia (Figs. 2 and 3) are borne on short hyaline pedicels. They are yellowish to light green when young and turn brown with age. They are spindle-shaped, somewhat curved, tapering at both ends, divided by four septa into a row of five cells, of which the three central are dark coloured while the other two form a kind of colourless cap at each end. From the lower end the persistent stalk on which the spore was borne projects as a slender tail. At the opposite end, the end cell grows out into three, rarely four, colourless thread-like appendages of considerable length ; these appendages very possibly help the dissemination of the spores by the wind.

The entire conidium measures from 11·7 to 28·3 μ in length and 3·3 to 6·7 μ in width on oat meal agar and 14·4 to 21·6 μ in length and 4·7 to 7·2 μ in width on leaves on the host. The appendages of the spores from oat meal agar vary in length from 4 to 28·3 μ and on the leaves from 7·2 to 25·2 μ.

V. Growth in Culture

The fungus was grown on oat meal, corn meal and potato-dextrose agars. About 20 hours after the transfers were made, white, rather coarse mycelial growth was observed on all media. An abundance of growth was noticed on oat meal agar and fairly abundant growth was observed on corn meal agar. On potato-dextrose agar the growth of the fungus was scanty. Generally the growth of the mycelium on all media was at first slow and creeping, then it became faster, until the mycelium was thick. The growth of the hyphæ was both aerial and submerged but on all substrata the aerial growth was more pronounced. Zonation was clear on all media. Two to three zones were noted on the growth of mycelium in 4 to 6 days. As the fungus grew old the growth in the first zone was usually covered with fruiting bodies. The mycelium then lost its fluffy appearance. Owing to the formation of numerous fruiting bodies the surface of the medium and substratum became covered with black, shiny, tar-like slimy masses of sporocarps.

The fruiting bodies are at first silver gray, somewhat resinous specks but later they turn tar black. The spores are produced five days after transfer on corn meal and potato-dextrose agars ; on oat meal agar the spores began to form in four days.

VI. Spore Germination

Spores were sown in distilled water and in a 5 per cent. glucose solution in drop cultures for germination study. In both these media the lowest of the three coloured cells was the germ-cell. This cell

swells, becomes nearly round and marked by a light ring round the middle and then puts out a (or rarely two) germ tube from the sides (Fig. 4).

Germination in distilled water was as follows. In 2½ hours few spores showed the germ cell becoming colourless and spherical, and after 4 hours' germ tubes 12 to 50 μ long and 1·5 to 2·5 μ wide were seen protruding from the sides. After 5 hours, one spore had 2 germ tubes coming out close to each other on the same side of the germ cell. After 24 hours, the majority of the spores had germinated, sending out generally one germ-tube which often branched at the base, but occasionally two, one on each side of the germ cell. The germ-tubes were sparingly branched and sparingly septate. In some cases a good number of spores had undergone no change while others had the germ cells spherical and swollen but had not germinated.

In 5 per cent. glucose solution early germ ination was more general and the growth of the germ tubes more luxuriant than in distilled water. After one hour in the culture medium the spores began to show signs of germination. The germ cell loses its colour and becomes spherical and swollen, increasing to about 8 to 9 μ in diameter. In a culture 4 hours' old the majority of the spores had germinated. The germ cell of some spores had swollen to a diameter of nearly 10 μ and had produced generally one germ tube, but sometimes two, one on each side of the germ-cell, non-septate and varying in length from 3 to 15 μ and in width from 2 to 4 μ. In a culture 24 hours' old the germ tubes were of varying length, much branched and septate, varying in width upto 6 μ.

VII. Temperature and Growth

The linear rate of growth of the fungus was studied on oat meal and potato-dextrose agars at various temperatures. The experiment was carried out in selected petri dishes of uniform size into which equal amounts of the medium were poured. All the dishes were inoculated at the same time and kept at various temperatures in darkness. The experiment was run in triplicate and repeated twice. The fungus was found to grow over a wide range of temperatures varying from 15° to 35° C. The fungus, however, grew well between 20° and 30° C. and temperatures above and below were detrimental to the growth of the fungus. It was also noticed that the optimum temperature for growth lies between 25° and 30° C.

VIII. Perpetuation and Dissemination

Intensive studies to determine the mode of perpetuation and dissemination of the parasite have not been undertaken. Limited studies and circumstantial evidence, however, have demonstrated that the parasite lives from one season to another, as mycelium and pycnidia within the tissues of the *tál* leaves. The leaves which dry and fall off owing to the disease serve as the resting place of the fungus. When conditions are favourable for its development the mycelium produces fruiting bodies the spores of which serve as inocula for primary infection.

In November 1942 pieces of naturally infected leaves were wrapped in tissue paper and carried through the rest of the winter and summer months under the following conditions :

(i) Hung on the tree in open.

(ii) Placed on the surface of the ground.

Isolations were made from these materials in October and November 1943 and the fungus was recovered in every instance. Inoculation experiments were carried out by using these isolations as inocula and in 95 per cent. cases infections were successful.

In the cultivation of *tál* sanitary methods are not at all followed. Diseased old leaves that have fallen are allowed to remain and rot in the ground nearby. Sometimes old leaves are allowed to remain hanging on the plant. In almost all localities one comes across piles of fallen leaves lying about. From these piles a large number of isolations were made during the years 1943 and 1944 beginning from November and ending in October next. In all cases the fungus was recovered. It can thus safely be concluded that the parasite perpetuates in the dead leaves lying near about *tál* plantations.

The parasite may be carried unintentionally by men from one place to another with green or dead leaves. Careful obseravations indicate that the parasite is disseminated through the agency of wind and rain.

IX. Control and Prevention

From these studies it will be evident that the disease can be controlled by systematic collection and destruction by burning of the affected leaves that lie on the ground or remain hanging on the trees and serve as a source of inoculum. Even slightly affected leaves should be severed from the plant and destroyed.

Preventive methods have been carried out with success. Spraying the plants with 2 : 2 : 50 Bordeaux resin-soda mixture at intervals of 15 days taking care to reach the youngest leaves have given promising results. It was found necessary that the spraying be done carefully so that the surface of the sprayed leaves were uniformly and thoroughly covered with the spray fluid.

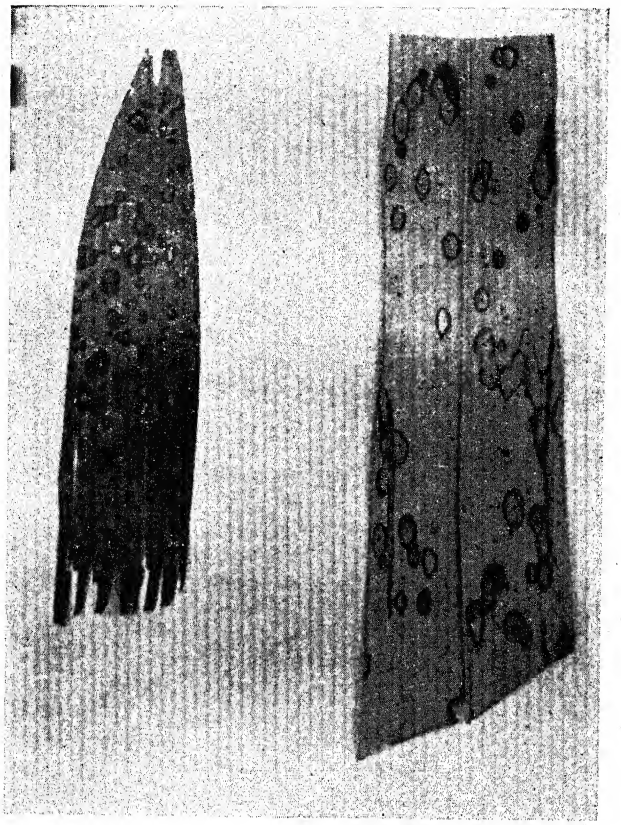
X. Summary

Pestalotia palparum Cke. causes a serious leaf spot of *Borassus flabellifer* in Assam. It impairs the value of the leaves as a material for the manufacture of hand fans and lowers the fruiting capacity of the plant.

Symptoms of the disease have been described.

Inoculation experiments carried out show that the fungus is a wound parasite and can infect only wounded surfaces of leaves. Cross inoculations have shown that the fungus can infect also the wounded leaves of *Areca catechu*, *Cocos nucifera* and *Phoenix sylvestris.*

The morphology of the parasite on the host as well as on culture has been described.



Symptoms of the disease

S. CHOWDHURY—

A LEAF SPOT OF BORASSUS FLABELLIFER *L.*
CAUSED BY PESTALOTIA PALMARUM *CKE.*

Growth on culture and spore germination have been studied and the details described in the text.

The fungus has been found to grow over a wide range of temperature, the optimum for growth lying between 25° and 30° C.

The parasite survives in the affected leaves lying in the ground and is disseminated by wind and rain.

The disease can be controlled by systematic collection and destruction of the affected leaves and prevented by spraying the plants with a Bordeaux resin-soda mixture.

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A NOTE ON THE DEVELOPMENT OF POLLEN OF *MYRISTICA FRAGRANS* VAN HOUTTEN AND THE AFFINITIES OF THE FAMILY MYRISTICACEÆ

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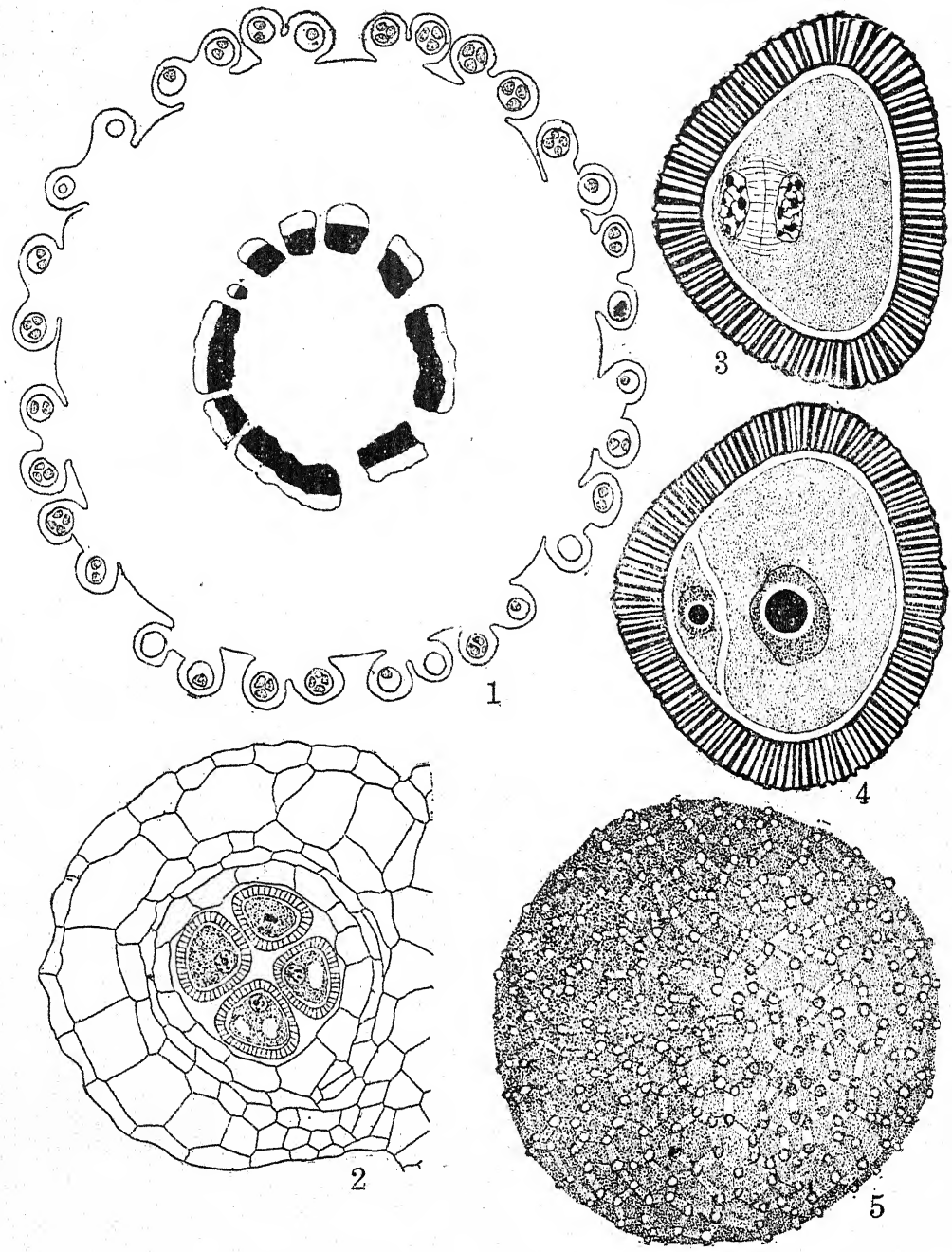
OUR knowledge of the embryological characters of the Myristicaceæ is very limited. Voigt (1888) long ago made a few observations on the structure of the ovule and endosperm of *Myristica fragrans* Van Houtten. He noted that the nucellus is well developed; the outer integument remains free from the inner right up to the chalaza, while the latter is fused with the nucellus in the lower half; and the ruminate endosperm probably develops according to the nuclear type. This is, however, the only information we have at present not only about this species but for the whole family Myristicaceæ.

Myristica fragrans is the well known nutmeg tree. The species is a native of the Molucca Islands, but it is now widely cultivated not only in the East Indies and Malay States, but also in Ceylon, West Indies and other tropical countries. The author during a short visit to Ceylon in December 1936 collected some material of the male flowers of this species from a tree growing in the Royal Botanic Garden, Peradenyia. This material did not include all the stages of development, but as nothing is known about the development of pollen and male gametophyte of the Myristicaceæ, it has been considered worthwhile to publish a brief account of the embryological characters that have been observed in this material.

THE MALE FLOWER

The male flowers are borne in lax, slender, supra-axillary racemes, 1–2 in. long. They are themselves about $\frac{1}{4}$ in. long, nodding, ellipsoid and bracteolate. The perianth is glabrate, 3-lobed and valvate. It has been shown previously (Joshi, 1943) that this trimerous perianth has been derived from a pentamerous whorl. The number of stamens in a male flower has been described by Hooker (1890) to vary from 9 to 12, but Fig. 1 shows that there are sometimes only 8 stamens. The stamens are connate and form a central cylindrical column. The lower part of this column is differentiated into a stalk-like structure, while the upper part bears anthers. The column is generally believed to have been derived from the fusion of filaments, but even the histological study does not reveal the individuality of the filaments (Fig. 1).



Figs. 1–5. *Myristica fragrans*. Fig. 1. Transverse section of the central column in the anther-bearing part showing the arrangement of the anthers and the vascular bundles. Fig. 2. Transverse section of an anther-lobe after the liberation of the pollen grains from the mother cells showing the structure of the anther wall. Fig. 3. A pollen grain in section showing the first division of the nucleus. Fig. 4. The same showing the formation of the generative cell. Fig. 5. A mature pollen grain in surface view.

Structure and Development of the Pollen

The anther wall consists of 5 layers of cells (Fig. 2), the epidermis, the fibrous endothecium, two middle layers which are crushed in later stages, and the tapetum. The last is of the secretory type. The tapetal cells remain uni-nucleate throughout their life, a condition which is comparatively rare among the angiosperm (see Cooper, 1933). The pollen mother-cells in an anther-lobe are arranged mostly in a single row. This fact remains clear for sometime even after the liberation of the pollen grains from the mother cells. The tetrads are isobilateral. Both in the arrangement of the pollen-mother cells in a single row in each anther-lobe and in the form of the pollen tetrads, *Myristica* agrees with many genera of the Anonaceæ, *e.g.*, *Anona*, *Xylopia* and *Monodora* (*cf.* Schnarf, 1931).

The first division of the pollen grain nucleus has been followed very clearly, particularly with regard to its polarity, as the pollen grains in this species do not round off immediately after their liberation from the mother cells and continue to occupy for sometime the same position that they had at the tetrad stage. The nucleus prior to this division shifts to the inner side and here it divides in a perfectly normal manner. The spindle organised during this division shows no unusual features (Fig. 3), as Hagerup (1938) has described in *Orchis*. The result of the division is the formation of a spindle-shaped generative cell towards the inner side of the pollen grain (Fig. 4) as Wulff and Maheshwari (1938) have already enumerated in *Symplocarpus*, *Xyris*, *Tradescantia*, *Apocyanum*, *Erica*, *Uvularia*, *Narcissus*, *Bulbine*, *Gasteria*, *Aloe* and *Clivia*. According to them, Geitler has reported the same condition also in many other genera of Liliaceous plants. A feature common to many of them is the presence of a single germinal furrow in the mature pollen grains. In this character the pollen grains of *Myristica fragrans* also agree.

The mature pollen grains are 2-nucleate and 2-celled, as Juliano (1935) and Locke (1937) have noted in *Anona* and *Asimina* respectively of the Anonaceæ. In fact, this character appears to be common to the Magnoliales in general (*cf.* Schnarf, 1939). The exine is reticulately marked and finely tuberculate (Fig. 5). Each mesh of the reticulum is usually pentagonal and at each angle of the pentagon there is a small tubercle. There is a single germinal furrow, as Wodehouse (1936) has noted in the Magnoliaceæ.

Discussion

In the last century there was a considerable difference of opinion with regard to the exact systematic position of the Myristicaceæ. Bentham and Hooker (1862–83), for example, placed it under Monochlamydeæ along with Piperaceæ, Chloranthaceæ and Monimiaceæ in the series Micrembryæ. It is evident that such a position is quite unnatural. In recent times, there has been a general agreement among taxonomists in assigning to Myristicaceæ a position in the neighbourhood of Ranunculaceæ, Magnoliaceæ, Anonaceæ, etc., but there are differences in details. Engler and Gilg (1924) place it in the order

Ranales, sub-order Magnoliineæ, which includes families like Menispermaceæ, Magnoliaceæ, Anonaceæ, Lauraceæ, etc. Bessey (1915) also places Myristicaceæ in his order Ranales. Wettstein (1924) includes it in his Reihe Polycarpicæ, which corresponds to the Ranales of the Englerian system. He places it next to the Anonaceæ and states it is related to that family in spite of the differences in floral structure. Hutchinson (1926), who has split the old order Ranales into several orders, places Myristicaceæ along with Monimiaceæ, Lauraceæ, Gamortegaceæ and Hernandiaceæ under Laurales. His Anonales includes only the families Anonaceæ and Eupomatiaceæ. Thus Hutchinson and Wettstein differ in their views about the exact affinities of the Myristicaceæ. While the latter considers it to be most nearly related to the Anonaceæ, the former believes that its nearest allies are to be found among the Lauraceæ.

The present study shows that the development of pollen in *Myristica fragrans* shows greatest resemblance with the Anonaceæ. This along with the presence of the ruminate endosperm appears to favour Wettstein's view about the close relationship of the two families Myristicaceæ and Anonaceæ.

Summary

The tapetum is of the secretory type. The tapetal cells remain uni-nucleate.

The pollen-mother cells in an anther-lobe are arranged in a single row.

The pollen tetrads are isobilateral.

The generative cell is cut off towards the inner side of the pollen grains.

The mature pollen grains are 2-celled and possess a single germinal furrow. The exine is reticulate and minutely tuberculate.

The evidence from microsporogenesis favours the hypothesis that the family Myristicaceæ is most closely related to the Anonaceæ.

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MODE OF INFECTION OF RICE BY *USTILAGINOIDEA VIRENS* (CKE) TAK.

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Introduction

Butler (1913) writes that the fungus *Ustilaginoidea virens* (Cke) Tak. was first described by Cooke in 1878 from Tinnevelly as *Ustilago virens*. He further mentions that Bredfeld studied the cultural behaviour of the fungus and established that it belongs to the genus *Ustilaginoidea* of the Ascomycetes.

In 1941, while examining diseased spikelets in the paddy fields at the Central Agricultural Experiment Station at Dacca, two different types of infected ears were noticed.

Since only a few ears are affected in the diseased plants and very often matured healthy rice grains are found closely associated with diseased ears, the author was encouraged to study the method of infection.

Distribution of the Disease

False smut of rice has been reported from most of the important rice-growing centres of the globe. Butler (1913) writes that it is found throughout India, more specially on the shores of the Bay of Bengal, in Malaya, Java, the Philippine Islands, China, Japan and the United States. Copeland (1924) describes the rice disease caused by *Ustilaginoidea virens* as green smut, and reports that it is always present in the Philippines and sometimes does considerable damage. Of the sixteen rice diseases reported from China, the green smut comes sixth in descending order of importance (Wei, 1934). Wei describes it as green smut instead of false smut. It has also long been reported from Japan, but did not receive much attention as it does not cause any serious loss to the crop. In Louisiana, even when the fungus is abundant, it does not cause even 1 per cent. damage to the crop. In Sumatra the appearance of green smut is taken as an indication of a crop fine in both quantity and quality.

False smut of rice is frequently seen in Bengal. Hedayetullah (1938) reports its appearance in some plots of paddy, *viz.*, Chinsura II, Nagra, and Patnai at Chinsura Farm and recommends the destruction of the affected plants for controlling the disease.

Butler and Bisby (1931) report it from Tinnevelly, Assam, Samalkota and Madras. It is also known from Bombay, Bihar and Orissa, but does not appear to have been observed so far in the Central Provinces

and the Punjab.* Bertus† reports that it occurs in Ceylon during the months of January and February. Su (1937) reports its appearance at Hwambi in Burma, and Mundkur (1940) collected it from Afganistan.

Material and Methods

Diseased materials in different stages of infection were examined and collected in 1941 and 1942 from *Aman* paddy (varieties : *Dadkhani*, *Kalamkati*, *Indrasail* and *Bhasamanik*) at the Central Agricultural Experiment Station at Dacca. The material was fixed in the field in form-acetic-alcohol. It was treated for varying periods with different concentrations of hydrofluoric acid to remove the silica from the glumes. The optimum concentration and period for hydrofluoric acid treatment were found to be :—(1) 20 c.c. hydrofluoric acid in 50 c.c. of 50 per cent. alcohol, and (2) 15–20 days.

For fixing the sections to the slides, Land's fixative substituting 'gloy' in place of gum arabic was used. The sections were stained in safranin and fast green, and iron-alum hæmatoxylin ; the former gave more satisfactory results.

Diseased materials in different stages of infection collected from the Konkan, Bombay Province, were also examined in 1944.

Symptoms of the Disease

Ustilaginoidea virens occurs as large, velvety green masses which vary from nearly 5 mm. to 8 mm. in diameter and are so conspicuous as to be noticeable from some distance. Spores are borne all over the surface of the infected region. In other respects the host plant appears to be quite normal and frequently attains the normal height of a healthy plant.

When sections of such sclerotial structures are examined it is observed that a compact mass of fungal hyphæ forms a sort of pseudo-parenchymatous tissue in the centre. Towards the periphery the hyphæ are more loosely arranged (Plate XIII, Fig. 1) and bear both terminal and lateral spores as previously observed by Butler (1913). Mature spores possess a very rough, granular, greenish brown coating and remain associated with the sporiferous hyphæ forming the outermost layer of the sclerotium. Spores which are somewhat younger and formed next to the outermost surface are almost brown in colour ; the youngest spores situated at the centre of the fungus ball are hyaline.

Nature of Infection

Diseased materials from several sources were examined and two types of infected panicles were observed. In the first type a few sclerotial structures are formed on the panicle in place of grains and most of the glumes of the spikelets are empty. In the second type very few sclerotial structures are found on the panicle and most of the

* Information supplied by Departments of Agriculture of these Provinces.

† Personal communication to Dr. P. Maheshwari.

glumes contain normal rice grains. In such cases some of the glumes containing mature grains are found to be infected with spores of *Ustilaginoidea virens* which remain associated with the hairs of the glume. These spores are fully matured and greenish brown in colour.

When empty glumes of the first type are thoroughly teased and examined it is observed that the anther lobes containing pollen grains as well as the feathery stigmas are infected with fungus mycelium (Plate XIII, Fig. 2). On removing the glumes and separating all parts of the spikelet it is seen that the flowers of rice plants are infected with the fungus at a very early stage before fertilization takes place. When such infected spikelets in different stages of infection are dissected and examined it is observed in every case that the essential organs of the flower, *i.e.*, the andrœcium and gynæcium are buried inside the central core of the pseudo-parenchymatous tissue of the fungus ball. The wall of anther lobes is found to be intact and pollen grains are preserved inside the anther lobes (Plate XIII, Fig. 3). Thus it appears that fertilization never takes place and as a consequence the rice grain is never formed in such cases.

Microtome sections (10 μ thick) of the fungus ball were examined with a view to studying the condition of stamens and the ovary, style and stigmas at a very advanced stage of infection. It seems that the ovary is disintegrated by the fungus, but the style, stigmas and anthers are preserved and buried inside the fungus ball (Plate XIII, Fig. 4). Pollen grains are invariably present inside the anther lobes.

Unlike the first type of infection, in the second type most of the glumes contain normal grains and only very few sclerotial structures are observed. Serial sections of several spikelets were cut to study the nature of infection in this case. In early stages of infection dense clusters of spores are seen along the two lines where the margins of the lemma and palea are interlocked with each other. In sections it appears as if a stream of these spores were proceeding inwards from these points with the purpose of entering into the grain (Plate XIII, Fig. 5). In a slightly later stage of infection the margins of lemma and palea are seen to separate so that some of these spores succeed in making their way through this opening (Plate XIII, Fig. 6). In a still later stage, due to a further gaping apart of the margins of the lemma and palea, a wide passage is formed and a large number of spores enter and come in contact with the pericarp of the fruit (Plate XIII, Fig. 7). Once the spores are there, they germinate and produce the mycelium in a comparatively short time.

The cells of the epidermis and mesocarp were found to be most susceptible to the attack of the fungus. In most cases it was observed that the cells of the epidermis and the mesocarp are disintegrated at a very early stage of infection while the cross-cells and the tube cells (belonging to the inner epidermis) still remain intact. In the next stage the cross-cells are also invaded. It is interesting to note that the inner epidermis formed by the tube cells is comparatively more resistant to the fungus. The moment the mycelium comes in contact with the endosperm its growth becomes greatly accelerated and ultimately

the whole grain is replaced by the fungus. The fungus ball continues to swell and the lemma and palea, the cells of which still remain free from fungus spores or mycelium, are pushed apart still further and thus a much wider gap is formed between their margins, as a result of which large numbers of spores enter into the grain from outside. In a very short time the fungus forms the green velvety mass and spores are formed from the mycelium and thrown out.

Discussion

A very striking feature of the false (or green) smut of rice is that the causal organism makes its appearance only on a few grains in the ear. Sections of stems, leaves and the inflorescence axes bearing diseased ears are seen to be absolutely free from the pathogen. Obviously the infection comes from outside.

From the materials studied so far and the description given above, it is obvious that there are two types of infection. In the first type the flower is invaded at a very early stage in its development which finally results in disintegration of the ovary, while the style, stigmas and the anther lobes containing pollen grains are entirely surrounded by the growing fungus which ultimately gives rise to a sclerotial structure, forming a pseudo-parenchymatous tissue, in the central core of which the style, stigmas and anther lobes are buried and preserved. It is difficult to explain why these parts of the essential organs of the flower are not disintegrated and destroyed by the fungus. In many cases the fungus fails to grow and develop and hence the characteristic fungus balls of green velvety appearance are formed only in a few infected spikelets. Hence in this type of infection fertilization does not take place and the grain is never formed.

In the second type of infection mature rice grains are infected. It appears that the spores are carried by the wind and some of them, by chance, adhere on the hairy surface of the glumes. The lemma and palea being very hard and rigid naturally offer a considerable amount of resistance to the entrance of the spores, and the latter can only enter through the passage between their margins. Spores accumulate in clusters near the marginal regions and the narrow cavity near the margins helps the spores in keeping themselves loosely fixed to the lemma and palea. These spores absorb moisture and swell and exert mechanical pressure due to which the margins of lemma and palea are forced apart forming an opening through which infection takes place. Since only a few grains are affected in the ear, it appears that only in those few cases the spores find their entry into the grain and gradually invade, and finally the whole tissue is replaced by the fungus ball.

Butler (1918) writes "the young ovary is invaded by the parasite at an early stage in its development" and his view closely agrees with the first type of infection described above. Butler (1918) also writes "the centre of the sclerotium is composed of pseudo-parenchyma entirely replacing the tissues of the grain", and this agrees with the second type of infection described in this paper. Hence invasion of young ovary by the parasite is the cause of the first type of infection,

while replacement of the tissues of the grain by the sclerotial structure is the result of the second type of infection.

Due to vigorous growth of the mycelium, the glumes are gradually pushed apart and a comparatively large space is formed near the margins of the glumes. The mycelium along with the spores protrudes out through these gaps, and finally the whole infected grain appears like a sclerotium which grows and bursts out between the two closely applied glumes. In a section of a fully infected grain, it is difficult to find out the two glumes which are very insignificant in size as compared to the size of the fungus ball (Plate XIII, Fig. 1).

SUMMARY

The symptoms of the false smut disease of rice caused by *Ustilaginoidea virens* (Cke) Tak. are described.

Two types of infection of the spikelets have been observed. In the first type, due to early infection of the flower, fertilization does not take place and the grain is never formed. In the second type the mature rice grain is infected by germinating spores of *Ustilaginoidea virens* and the grain is finally replaced by the fungus ball.

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EXPLANATION OF THE PLATE

Fig. 1. One half of *t.s.* of a fully infected spikelet; *g*, glume; *an*, *t.s.* of anther; *h*, loosely arranged hyphæ towards the periphery (×83).

Fig. 2. Anther containing pollen grains and feathery stigma infected with mycelium of *Ustilaginoidea virens* (×120).

Fig. 3. *t.s.* of an infected spikelet showing an anther containing pollen grains embedded in the pseudo-parenchymatous tissue of the fungus ball (×300).

Fig. 4. Section of a fully infected spikelet showing: *st*. style; *fs*. feathery stigma; *an*, *t.s.* and *l.s.* of anther embedded in the pseudo-parenchymatous tissue (×83).

Fig. 5. Spores of *U. virens* proceeding towards the regions where margins of the glumes are interlocked with each other (×249).

Fig. 6. Margins of the glumes gaping apart and some of the spores succeed in entering through the opening (×249).

Fig. 7. Spores in direct contact with the pericarp of the fruit; *sp*, cluster of spores; *p*, pericarp (×300).

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